BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. CONKLIN—*Princeton University.*

GEORGE T. MOORE—*The Missouri Botanic Garden.*

T. H. MORGAN—*Columbia University.*

W. M. WHEELER—*Harvard University.*

E. B. WILSON—*Columbia University.*

Managing Editor

FRANK R. LILLIE—*The University of Chicago.*

VOLUME XLV.

WOODS HOLE, MASS.

JULY TO DECEMBER, 1923

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VOL. XLV JULY, 1923 NO. 1

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Managing Editor

FRANK R. LILLIE—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa.

Vol. XLV. *July, 1923.* *No. 1*

BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY.

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I. TRUSTEES.

EX OFFICIO.

FRANK R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
*D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.
CORNELIA M. CLAPP, Mount Holyoke College, EMERITUS.

TO SERVE UNTIL 1926.

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OTTO GLASER, Amherst College.
ROSS G. HARRISON, Yale University.
H. S. JENNINGS, Johns Hopkins University.
F. P. KNOWLTON, Syracuse University.
M. M. METCALF, Oberlin, Ohio.
WILLIAM PATTEN, Dartmouth College.
W. B. SCOTT, Princeton University.

*Deceased.

1

TO SERVE UNTIL 1925.

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I. F. LEWIS, University of Virginia.
R. S. LILLIE, Nela Research Laboratory.
E. P. LYON, University of Minnesota.
C. E. MCCLUNG, University of Pennsylvania.
T. H. MORGAN, Columbia University.
D. H. TENNENT, Bryn Mawr College.
E. B. WILSON, Columbia University.

TO SERVE UNTIL 1924.

H. H. DONALDSON, The Wistar Institute of Anatomy and Biology.
W. E. GARREY, Tulane University.
CASWELL GRAVE, Washington University.
M. J. GREENMAN, The Wistar Institute of Anatomy and Biology.
*GEORGE LEFEVRE, University of Missouri, *Secretary of the Board.*
A. P. MATHEWS, The University of Cincinnati.
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C. R. STOCKARD, Cornell University Medical College.

TO SERVE UNTIL 1923.

H. C. BUMPUS, Brown University.
R. A. HARPER, Columbia University.
W. A. LOCY, Northwestern University.
JACQUES LOEB, The Rockefeller Institute for Medical Research.
GEORGE T. MOORE, Missouri Botanical Garden, St. Louis.
L. L. NUNN, Telluride, Colorado.
W. J. V. OSTERHOUT, Harvard University.
WILLIAM M. WHEELER, Bussey Institution, Harvard University.

II. ACT OF INCORPORATION.

No. 3170

COMMONWEALTH OF MASSACHUSETTS.

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintain-

*Deceased.

ing a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand, Eight Hundred and Eighty-Eight.

[SEAL] HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY.

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees

to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee of finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT.[1]

Harvey S. Chase & Company,
Certified Public Accountants, 84 State Street, Boston.

January 22, 1923.

Mr. D. Blakely Hoar,
161 Devonshire Street,
Boston.

Dear Sir: We have completed our audit of the accounts of the Marine Biological Laboratory for the year ended December 31, 1922, as kept both at your office in Boston and at Woods Hole, and report thereon in the accompanying exhibits and schedules:

Exhibit A—Balance-Sheet as of December 31, 1922.

Exhibit B—Income-and-Expense for the Year ended December 31, 1922.

Schedule I—Investments (Book Values).

Schedule II—Cash Receipts and Disbursements on Account of Funds.

Schedule III—Land, Buildings, and Equipment.

Schedule IV—Supply Department Income-and-Expense Account for the Year ended December 31, 1922.

We certify that, subject to the comments herewith, the balance-sheet and income-and-expense statement shown in Exhibits A and B are in accordance with the books and correct, to the best of our knowledge and belief.

Very respectfully,

Harvey S. Chase & Company,
Certified Public Accountants.

[1] Only a part of the audit is included in the Treasurer's Report. The complete audit is on file at the Laboratory and may be examined by any member.

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1922.

Assets.

Cash:		
In bank	$ 1,592.05	
Petty cash fund	200.00	$ 1,792.05
Accounts receivable		14,535.47
Inventories:		
Supply department	23,960.63	
BIOLOGICAL BULLETIN	3,329.25	27,289.88
Investments:		
Securities—Schedule I	12,151.12	
Less Loan	1,100.00	
	11,051.12	
Cash—Schedule II	516.64	11,567.76
Stock in General Biological Supply House, Inc.		12,700.00
Gansett property account	18,832.22	
Less mortgage	10,782.01	8,050.21
Educational Plant—Schedule III:		
Land	95,856.14	
Buildings	209,183.50	
Equipment	99,430.61	
	404,470.25	
Less Reserve for Depreciation	51,050.52	353,419.73
Items in suspense:		
First payment on purchase of Fish Estate, Woods Hole	5,000.00	
Expense for surveying and testing ground in re proposed extension of laboratory building	418.49	
Sundry items	32.16	5,450.65
Prepaid insurance		1,304.85
		$436,110.60

Liabilities.

Accounts payable		$ 2,611.66
Notes payable—Falmouth National Bank		10,000.00
Accrued charges (estimated)		1,200.00
Items in suspense		59.30
Trust funds		11,567.76
		$ 25,438.72
Balancing Account:		
Balance, January 1, 1922	$399,777.13	
Add:		
Balance of income account for year	5,894.75	
Special donation received from C. R. Crane for first payment on purchase of Fish Estate	5,000.00	410,671.88
		$436,110.60

MARINE BIOLOGICAL LABORATORY, INCOME & EXPENSES FOR YEAR ENDED DECEMBER 31, 1922.

	Expenses	Income	Loss	Gain
Administration expenses	$ 9,570.85		$ 9,570.85	
Bar Neck Property expense	318.00		318.00	
BIOLOGICAL BULLETIN and annual dues	4,265.50	$ 3,653.31	612.19	
BIOLOGICAL BULLETIN, adjustment of expenses for 1921	200.21		200.21	
Carpenter department	958.18	1.55	956.63	
Chemical department	2,012.79		2,012.79	
Dormitories	2,384.01	2,660.78		$ 276.77
Instruction	6,866.46	9,390.00		2,523.54
Interest on notes payable	700.66		700.66	
Janitor's house expense	7.84		7.84	
Library department	1,860.22		1,860.22	
Maintenance, buildings and grounds	6,640.42		6,640.42	
Mess	24,328.81	26,184.05		1,855.24
New laboratory	4,276.30		4,276.30	
Newman cottage	80.13	150.00		69.87
Pumping station	587.39		587.39	
Research department	2,817.16	6,425.00		3,607.84
Sundry expense and income	804.11	10,853.61		10,049.50
Supply department (See Schedule IV)	44,004.65	49,368.07		5,363.42
Truck	783.14		783.14	
Total current expenses	$113,466.83		$28,526.64	
Total current income	108,686.37	$108,686.37	23,746.18	$23,746.18
Excess of expenses	4,780.46		4,780.46	
Reserve for depreciation	8,980.35			
Bad accounts written off	365.97			
	$14,126.78			
Donations for expenses:				
Friendship Fund Inc. $20,000.00				
Others 21.53	20,021.53			
Balance to balancing account	$ 5,894.75			

MARINE BIOLOGICAL LABORATORY INVESTMENTS, DECEMBER 31, 1922.

Reserve Fund.

Cash on hand	$ 388.41	
* $3,000.00 American Telephone & Telegraph Company, 4's	2,921.25	
500.00 Western Telephone & Telegraph Company, 5's	496.88	
* 6 shares American Smelting & Refining Company, Preferred	732.00	
8 shares General Electric Company	907.25	
4 shares General Electric Company Special (par $10.00) received as a stock dividend.		
14 shares United Shoe Machinery Corporation, Preferred	393.75	
5 shares Massachusetts Gas Companies, Preferred	444.63	
	$6,284.17	
Items marked * are held as collateral on loan of	1,100.00	$5,184.17

Library Fund.

$300.00 U. S. Liberty Loan, First 4 1/4's	300.00	
4/5 of $1,000.00 American Telephone and Telegraph Company, 4's	779.00	
3 shares American Telephone & Telegraph Company	362.38	
3 shares General Electric Company	346.80	
1 1/2 shares General Electric Company Special (par $10.00) received as stock dividend.		
5 shares United Shoe Machinery Corporation, Preferred	140.63	
3 shares Massachusetts Gas Companies, Preferred	269.38	
*1 share American Smelting & Refining Company, Preferred	122.00	
	$2,320.19	
Less overdraft of cash	120.88	
		2,199.31

Lucretia Crocker Fund.

Cash on hand	249.11	
$300.00 U. S. Liberty Loan, 1st 4 1/4's	300.00	
1/5 of $1,000.00 American Telephone & Telegraph Company, 4's	194.75	
18 shares Vermont & Massachusetts Railroad Company	2,416.50	
3 shares General Electric Company	349.55	
1 1/2 shares General Electric Company Special (par $10.00) received as stock dividend.		
1 share Boston Elevated Second Preferred	133.00	
1 share American Telephone & Telegraph Company	120.79	
4 shares Boston Consolidated Gas Company, Preferred	420.58	4,184.28
Total, Exhibit A		$11,567.76

V. REPORT OF THE LIBRARIAN.

The growth of the Library continues steadily. The number of books added during the year was 473. Of these eighty volumes were received by purchase, 199 by binding of periodicals, 157 were gifts, and 21 were additions to the permanent loan from the American Museum of Natural History. Our valuable collection of reprints has been increased by the addition of 434 pamphlets.

The number of periodicals currently received was 229; of which 87 are received by subscription, 59 by exchange for the Biological Bulletin, 69 were gifts, and 14 were duplicates lent to us by the American Museum of Natural History. The total contents of the Library at the end of the year is 11,136 volumes and 9,393 pamphlets.

Two of the gifts received during the year are especially noteworthy. The first to be mentioned is the gift from Mrs. George Peirce of books selected from the working library of her husband, a distinguished physiologist and chemist, whose herioc death in 1919 at the height of a productive career of research has retarded the advance of biochemistry in America. This is a collection of thirty volumes, all recent books of importance, and is a very valuable addition to our library.

The second gift of special note is a complete set of the cards of the Concilium Bibliographicum. This is given by the Library of Carleton College through the good offices of Dr. Donnell B. Young of our Instructing Staff. These cards will be of very great use in the bibliographic research that is a prominent part of the work of investigators every summer. But before they can be used these cards must be filed in a suitable cabinet. Until a cabinet and room for it can be provided, the cards can only be stored.

Another notable gift is a collection of thirteen volumes of their publications given by Messrs. P. Blakiston's Son & Company through their representative, Mr. Horace G. White. These books, with one exception, are all published in 1919, or later, and all are desirable additions to the Library.

Dr. Christine Ladd-Franklin has presented to the Library the Concise Oxford Dictionary, edition of 1911, and a copy of Woodworth's Psychology, 1921. From Dr. R. P. Bigelow have been received several volumes, including the U. S. Entomological Commission Reports, Volumes 2 and 3, 1878–82.

Through the kindness of their authors we have received the following books:

B. M. Patton, "Laboratory Directions in Embryology," 5 volumes.

E. G. Conklin, "Direction of Human Evolution."

G. H. Parker, "Smell, Taste, and Allied Senses in Vertebrates."

W. C. Curtis, "Science and Human Affairs."

L. L. Woodruff, "Foundations of Biology."

B. H. Grave, "Birds of Wyoming."

W. A. Collier, "Einführung in die Variationsstatistik."

H. C. van der Heyde, "Physiology, Digestion, Respiration, and Excretion in the Echinoderms."

J. F. Nonides, "Herencia Mendeliana."

Libbie H. Hyman, "Laboratory Manual of Comparative Vertebrate Anatomy."

C. T. Brues, "Insects and Human Welfare."

A. H. Church, "Thallassophyta and Sub-aërial Transmigration."

When the present officers took over the administration of the Library, there was found a large accumulation of printed matter, mostly unbound, that was thought to be duplicates. During the early part of the year covered by this report, the Assistant Librarian spent considerable time sorting this material. Thirty-three volumes were found not to be duplicates and were added to the Library. They are included in the gifts mentioned in the first paragraph of this report. From the sale of 450 duplicates, we received $106.20, which was applied to the purchase of new books. There remain about 150 odd numbers and volumes and 1,000 reprints still to be catalogued.

The Director having authorized an extension of the exchange list, considerable time has been given to selecting and soliciting new exchanges with the Biological Bulletin. Nineteen new exchanges have been established, some of them bringing more

than usually desirable publications, and in the following cases this includes back sets as well as current issues:

Hereditas,
Acta Zoologica,
Annalen des Naturhistorischen Museums, Vienna,
Archives Néerlandaises de Physiologie,
Carlsberg Laboratorium, Comptes Rendus,
Skandinavisches Archiv für Physiologie,
Svensk Botanisk Tidskrift, Vol. 1, 1907, to 16, 1922,
Kolloid Zeitschrift,
Revue générale des sciences pures et appliquées.

The completion of our sets of serial publications is one of the important aims of the library administration. It is, therefore, gratifying to report that we have been able to complete the following by purchase:

Allgemeine botanische Zeitschrift, Vols. 1–25,
Botanische Jahrbücher, Vols. 1–56,
Journal of Pharmacology and Experimental Therapeutics, Vols. 1–11,
Zeitschrift für Botanik, Vols. 1–13,
Zoological Record, Vols. 31–57.

The dictionary catalogue which was begun in 1920, now contains 11,051 cards, and work has started on the transfer of cards from the old classified catalogue.

The increase in the use of the Library has continued, the circulation during 1922 being about 1,200 items, besides 30 volumes borrowed from other libraries.

We still lack many reprints of work done at Woods Hole, and authors are requested to look over the reprints in the Library and to send us what are lacking. Among the special needs of the Library are a complete set of the *Philosophical Transactions of the Royal Society*, a good encyclopedia, and bibliographical aids, such as the Royal Society Catalogue of Scientific Papers, and several sections of the International Catalogue of Scientific Literature.

ROBERT P. BIGELOW,
Librarian.

VI. THE DIRECTOR'S REPORT.

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY.

Gentlemen: I beg to submit herewith a report of the thirty-fifth session of the Marine Biological Laboratory for the year 1922.

The business of the Laboratory has become so complex, especially in the summer, that the executive officers can no longer assume the responsibility of administering it with reference only of major problems to the Board of Trustees once or twice during the year. The Executive Committee of the Board has accordingly been reorganized during the last two years to consist of the Director and Assistant Director *ex officiis* and three members of the Board resident in Woods Hole during the summer, one member being elected each year for a three-year period. The Committee meets weekly during the summer, and at other times of the year when called, and reports its principal activities at the annual meeting of the Board. In this way different members of the Board are successively brought into close contact with detailed problems of administration of the Laboratory. It is felt that this practice is good for the morale of the entire Institution; it is also a great relief to the administrative officers to share their responsibilities at times when decisions have to be made; naturally, also, it makes for greater steadiness and consistency in the conduct of affairs, through the establishment of precedents.

The season of 1922 was very gratifying as to attendance and all other evidences of active interest and wide coöperation in the affairs of the Laboratory.

Attendance.—The attendance of students in courses was 126, of investigators 182, making a total of 308 persons, who represented 104 institutions. These numbers are considerably in excess of any previous attendance, except in the case of students in courses whose numbers are limited by rule. Applications for working places were very considerably greater than could be granted; about 30 students had to be refused places on account of the rule limiting admission to courses, but it was pos-

sible to provide for nearly all applicants for research facilities. There is no doubt that if the present limitations in the Laboratory were removed, that numbers would mount much higher. For an institution devoted primarily to research, as ours is, this cannot be regarded as an end in itself. But there is no doubt that the present accommodations are inadequate to fulfill the present strictly justifiable needs of American Biology. This becomes more obvious year by year. If we are to continue to justify the status of the Marine Biological Laboratory as the national center of biological research we must accommodate ourselves to the idea of a future considerable increase in attendance. The investigator who desires quiet for his research must use the same rules of protection in the Marine Biological Laboratory that he does in the still larger university communities from which he generally comes.

The number of subscribing and coöperating institutions increased from 52 in 1921 to 61 listed on page 30; there were in addition three scholarship arrangements providing for five students. Of the total amount of $9,687.50 received from these institutions, $3,912.50 went for students' tables and $5,775.00 for research accommodations. In 1921 the receipts from subscribing and coöperating institutions amounted to $8,800.00. As 58 per cent. of the students paid their own fees in 1922 the fear that has been expressed lest subscribing and coöperating institutions should monopolize all the students' places does not appear to be justified. This is borne out in another way by comparing the total of institutions represented, 104, with the number of coöperating and subscribing institutions, 61. The growth in the number of these institutions is an encouraging sign of continued interest which represents the broad basis of support of the Laboratory.

The Report of the Treasurer (see p. 5).—The earned income of the Laboratory increased from $98,292.38 in 1921 to $108,686.37 in 1922; and the current expenses increased from $109,970.40 in 1921 to $113,466.83. The excess of current expenses over earned income was $11,678.02 in 1921 and only $4,780.46 in 1922. If it were not for the constant need for additions out-

side of current expenses the Laboratory would be approaching a self-supporting basis. The annual subvention of $20,000.00 a year from the Friendship Fund provides both for the excess of current expenses and also for necessary annual improvements and additions. The net assets of the Laboratory increased from $430,251.28 in 1921 to $436,110.60 in 1922; and the liabilities decreased from $30,474.15 in 1921 to $25,438.72 in 1922.

The increase of income over 1921 amounting to $10,393.99 is due to a relatively slight increase of the receipts of all departments and a large increase in the income of the Supply Department from $42,774.78 in 1921 to $49,368.07 in 1922.

Great encouragement in our plans for the new buildings proposed since 1919 was received during the year from the Rockefeller Foundation, from the Carnegie Corporation, and from Mr. C. R. Crane. Early in January the Board of the Rockefeller Foundation voted to authorize negotiations with the Marine Biological Laboratory on the basis of one-half of the total sum called for in our estimates, viz.: $1,000,000 for completing the Laboratory, library, and auditorium building and providing endowment for its maintenance. This was conditioned upon the total sum being raised, the continuance of the sum of $20,000 annually from the Friendship Fund, and making of satisfactory arrangements for reversion of endowment in case the work of the Laboratory were to be radically changed or abandoned. In February the Trustees of the Carnegie Corporation appropriated the sum of one hundred thousand dollars ($100,000) to the Marine Biological Laboratory for the purpose of creating a fund the income of which shall be used for the maintenance of its buildings and laboratories provided gifts to the total amount of one million dollars ($1,000,000) be secured for the joint purpose of providing for buildings and for their maintenance. Mr. Crane also assured the Director that the maintenance of $20,000 a year from the Friendship Fund would be guaranteed as soon as the other conditions were met. Circumstances during the remainder of the year did not permit an active campaign to secure the additional funds needed though inquiries have been made that should inform us during the session of 1923 just what efforts will be required.

During the summer farther study of the building plans and of equipment was made and incorporated in working drawings and specifications by the architects. Costs of building having increased since the original estimates were prepared in March 1922, the necessity of some curtailment of building plans was considered, and alternative schemes for constructing major parts of the entire plan were also prepared by the architects. The plans and specifications are ready for the builders as soon as the balance of the sum needed to meet the conditions has been provided.

The Laboratory traces its origin directly back to the impetus given the study of Marine Biology by the establishment of the Anderson School of Natural History on Penikese Island near Woods Hole in 1873 by Louis Agassiz. As the summer of 1923 will be the fiftieth anniversary of this epoch-making event in the history of biology in America, it seemed to be an appropriate time for the establishment of a memorial in memory of this service of Agassiz. On recommendation of Dr. Clapp a committee was appointed by the Trustees to consider and recommend plans for the establishment of an Agassiz Memorial. It is hoped that this may take the form of a suitable monument on the site of Agassiz's laboratory.

Towards the end of the year a most important addition to the real estate holdings of the Laboratory was made by the President of the Board, Mr. C. R. Crane, who presented to the Laboratory the property known as the Kidder House at the corner of East and Water Streets immediately adjoining the old buildings of the Laboratory and forming part of the block on which the first buildings of the Laboratory were erected. We owe a debt of gratitude to Mrs. Kidder as well as to Mr. Crane for the acquisition of this land, which she may be said to have held for the Laboratory pending a favorable opportunity for its acquisition. In the Resolution concerning the death of Mr. Camillus G. Kidder, included in this report, reference is made to the interest of the Kidder family in plans for biological work at Woods Hole at a period even ante-dating the establishment of the Marine Biological Laboratory. It is to that interest

that we owe our most important sites, including the land on which the Crane laboratory stands; the present status of the Laboratory is in an important respect the fruition of the far-sightedness of the Kidder family.

The Trustees at their meeting on August 8, 1922, established a class of emeritus trustees to honor Dr. Cornelia M. Clapp who expressed her desire to be relieved from membership on the Board after twenty-two years of service. This action was taken in acknowledgment of the exceptional spirit of devotion displayed toward the laboratory from its foundation to the present time by Miss Clapp and in the confident expectation of her continued interest. Dr. Clapp was accordingly elected Trustee Emeritus at the meeting of the Board in August 1922.

At the meeting of the Corporation August 8, 1922, two new members were elected to the Board of Trustees to fill vacancies in the newly elected class of 1926: Professor Otto Glaser of Amherst College, and Professor F. P. Knowlton of Syracuse University, both old members of the Corporation and regular investigators at the Marine Biological Laboratory.

At the meeting both of the Trustees and also of the Corporation the deaths of three former Trustees of the Laboratory were commemorated in the following memorials:

Resolution on the death of William T. Sedgwick, drawn up by Dr. Cornelia M. Clapp:

William T. Sedgwick, professor of biology and public health at the Massachusetts Institute of Technology, Boston, died on January 25, 1921.

The career of Professor Sedgwick as a teacher is well known. He was a fluent lecturer, and as one of his students has said he could make science popular and could take subjects of popular interest and clothe them in the language of science. Soon after he came to Boston he became consulting biologist of the State Board of Health and after that was identified with the interests of the American Public Health Association. He has been called the "Ambassador of Health." It is, however, as a member of the Board of Trustees of the Marine Biological Laboratory that we remember him today. In 1887 came an awakening of

interest in a project for starting a sea-side laboratory, and his aid was sought by the Woman's Education Association in their efforts in this direction.

It was proposed that there should be a permanent institution, incorporated, and supported by the educational institutions of the country. There is no name more prominent in this movement than that of Professor Sedgwick. He was one of the original seven trustees, served on committees, suggested and helped carry out plans for raising funds, enlisted the aid of benevolent Bostonians, and not least among his activities was the effort to master the practical details concerned with the living conditions in Woods Hole.

He spent the summer of 1888 in this vicinity looking after the interests of the Laboratory. His devotion to the Laboratory during the earliest and most critical period of its history will be remembered and cherished by the friends of the Laboratory. A life-long friend, Dr. E. B. Wilson, has written an appreciation which it is hoped will find a place among the records of the Marine Biological Laboratory.

Resolution on the death of Camillus G. Kidder, drawn up by Dr. E. B. Wilson:

The Trustees of the Marine Biological Laboratory record with deep regret the death of Camillus G. Kidder, a member of this Board since 1897, and always one of its highly valued friends and loyal supporters. Both Mr. Kidder and his brother, Dr. Jerome H. Kidder, were among the earliest of the summer residents at Woods Hole, and both were from the beginning in close touch with the biological work here carried on, through their close friendship with Spencer F. Baird and his associates in the U. S. Fish Commission. From the first Mr. Kidder showed a sympathetic interest in Baird's plans for the larger development of that work. A conspicuous example of this, and one that should have a place in the annals of the Marine Biological Laboratory, was the early purchase by himself and Dr. Kidder, at Baird's suggestion, of the land on which the new laboratory now stands, in order to hold it in friendly hands with a view to the possible later development of Baird's plans. When

the Marine Biological Laboratory was established at Woods Hole in 1888, Mr. Kidder unhesitatingly extended his interest to the new enterprise, becoming a steadfast supporter of its plans for scientific work and for friendly coöperation with the Fish Commission. Years later it was through him and Mrs. Jerome Kidder that Baird's foresight brought fruit through the acquisition by the Marine Biological Laboratory of the Kidder Land, later to become the site of the Crane Laboratory, and the future site, as we hope, of its further extension.

The Trustees here record their appreciation of Mr. Kidder's wise counsel in the conduct of the Laboratory and of the kindly and understanding spirit in which he took part in the deliberations of this Board and from time to time presided over its meetings. We cherish the memory of his sympathetic personality and generous friendship, and we are grateful for the long continued services that he rendered.

Resolution on the death of Alfred G. Mayor, drawn up by Dr. E. G. Conklin:

The death of Dr. A. G. Mayor, Director of the Department of Marine Biology of the Carnegie Institution of Washington at his laboratory at Tortugas, Florida, on June 24 last is lamented as a serious loss by the Marine Biological Laboratory. Dr. Mayor was not only a distinguished organizer and director of research in tropical marine biology and the leading American student of Cœlenterata but he was ever the loyal friend of the Marine Biological Laboratory. He carefully planned the work of his Department of the Carnegie Institution so as to supplement and not to duplicate or interfere with the work of this Laboratory, and at a critical period in our history he said that he would gladly abandon his cherished projects if by so doing he could materially aid the Marine Biological Laboratory. He was a useful member of our Board of Trustees and he assisted in the work of our institution not only by his attendance and lectures but also by furnishing facilities and generous assistance for investigations in tropical waters to members of our Staff and Corporation.

It is especially as a helpful, unselfish, and genial friend that

we love to remember him; he was always ready to sacrifice himself for the good of others and it can be truly said that he gave his life not merely to science but also to his fellow scientists.

The Marine Biological Laboratory expresses to his family its deep sympathy in their bereavement and its high appreciation of his work and character.

There is attached as part of this report a list of the Staff and of Investigators and Students for 1922, a tabular view of attendance 1918–1922, lists of subscribing institutions, of the Evening Lectures and of the Members of the Corporation.

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1. THE STAFF.

1922.

FRANK R. LILLIE, *Director*, Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.

ZOÖLOGY.

I. INVESTIGATION.

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.

E. G. CONKLIN, Professor of Zoölogy, Princeton University.

CASWELL GRAVE, Professor of Zoölogy, Washington University.

H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.

GEORGE LEFEVRE, Professor of Zoölogy, The University of Missouri.

FRANK R. LILLIE, Professor of Embryology, The University of Chicago.

C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.

S. O. MAST, Professor of Zoölogy, Johns Hopkins University.

T. H. MORGAN, Professor of Experimental Zoölogy, Columbia University.

G. H. PARKER, Professor of Zoölogy, Harvard University.

E. B. WILSON, Professor of Zoölogy, Columbia University.

II. INSTRUCTION.

ROBERT H. BOWEN, Instructor in Zoölogy, Columbia University.

EDWARD F. ADOLPH, Instructor in General Physiology, University of Pittsburgh.

J. A. DAWSON, Assistant Professor of Biology, Dalhousie University.

ANN H. MORGAN, Professor of Zoölogy, Mount Holyoke College.

C. L. PARMENTER, Instructor in Zoölogy, University of Pennsylvania.
J. PAUL VISSCHER, Instructor in Zoölogy, Washington University.
BENJAMIN P. YOUNG, Assistant Professor of Zoölogy, Cornell University.
DONNELL B. YOUNG, Assistant Professor of Biology, Carleton College.

PROTOZOÖLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
LOUISE H. GREGORY, Assistant Professor of Zoölogy, Columbia University. (Absent on leave.)
FLORENCE DEL. LOWTHER, Instructor in Zoölogy, Barnard College.

EMBRYOLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

HUBERT B. GOODRICH, Associate Professor of Zoölogy, Wesleyan University.
BENJAMIN H. GRAVE, Professor of Biology, Wabash College.
ROBERT S. MCEWEN, Assistant Professor of Zoölogy, Oberlin College.
HAROLD H. PLOUGH, Associate Professor of Biology, Amherst College.
CHARLES G. ROGERS, Professor of Comparative Physiology, Oberlin College. (Absent on leave.)
ELIZABETH A. SMITH, Assistant Professor of Zoölogy, University of Wisconsin.

PHYSIOLOGY.

I. INVESTIGATION.

HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.
WALTER E. GARREY, Professor of Physiology, Tulane University.
RALPH S. LILLIE, Biologist, Nela Research Laboratory, Department of Pure Science, Nela Park, Cleveland, Ohio.
ALBERT P. MATHEWS, Professor of Biochemistry, The University of Cincinnati.

II. INSTRUCTION.

MERKEL H. JACOBS, Assistant Professor of Zoölogy, University of Pennsylvania.
FRANK P. KNOWLTON, Professor of Physiology, Syracuse University.

ALFRED C. REDFIELD, Assistant Professor of Physiology, Harvard Medical School.

REYNOLD A. SPAETH, Associate in Physiology, School of Hygiene and Public Health, Johns Hopkins University.

BOTANY.

I. INVESTIGATION.

S. C. BROOKS, Department of Public Health, Washington, D. C.

EDWARD M. EAST, Professor of Experimental Plant Morphology, Harvard University.

ROBERT A. HARPER, Professor of Botany, Columbia University.

E. NEWTON HARVEY, Assistant Professor of Physiology, Princeton University.

WINTHROP J. V. OSTERHOUT, Professor of Botany, Harvard University.

II. INSTRUCTION.

IVEY F. LEWIS, Professor of Biology, University of Virginia.

WILLIAM RANDOLPH TAYLOR, Instructor in Botany, University of Pennsylvania.

WILLIAM H. WESTON, JR., Assistant Professor of Cryptogamic Botany, Harvard University.

LIBRARY.

ROBERT P. BIGELOW, Librarian and Associate Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology. Librarian.

PRISCILLA B. MONTGOMERY (Mrs. Thomas H. Montgomery, Jr.), Assistant Librarian.

CHEMICAL SUPPLIES.*

OLIVER S. STRONG, Associate Professor of Neurology, Columbia University, New York City, Chemist.

SUPPLY DEPARTMENT.

GEORGE M. GRAY, Curator.

THOMAS M. DOUTHART, Assistant Curator.

JOHN J. VEEDER, Captain.

E. M. LEWIS, Engineer.

A. W. LEATHERS, Head of Shipping Department.

A. M. HILTON, Collector.

J. MCINNIS, Collector.

F. M. MACNAUGHT, Business Manager.

2. INVESTIGATORS AND STUDENTS, 1922.

INDEPENDENT INVESTIGATORS—Zoölogy.

ADAMS, A. ELIZABETH, Associate Professor of Zoölogy, Mount Holyoke College.
ADDISON, WILLIAM H. F., Professor of Histology and Embryology, University of Pennsylvania.
ADOLPH, EDWARD F., Instructor in Physiology, University of Pittsburgh.
ANDERSON, ERNEST G., Research Associate, Carnegie Institution, Cold Spring Harbor.
BASCOM, KELLOGG F., University of Chicago.
BIGELOW, ROBERT P., Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology.
BISHOP, MABEL, Professor of Zoölogy, Hood College.
BODINE, JOSEPH H., Instructor in Zoölogy, University of Pennsylvania.
BOWEN, ROBERT H., Instructor in Zoölogy, Columbia University.
BRIDGES, CALVIN B., Research Assistant, Carnegie Institution of Washington.
BUDINGTON, ROBERT A., Professor of Zoölogy, Oberlin College.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CAROTHERS, E. ELEANOR, University of Pennsylvania.
CHAMBERS, ROBERT, Professor of Histology and Embryology, Cornell University Medical College.
CHARLTON, HARRY H., Assistant Professor of Anatomy, University of Missouri.
CLAPP, CORNELIA M., Professor Emeritus of Zoölogy, Mount Holyoke, College.
CLARK, ELIOT R., Professor of Anatomy, University of Missouri.
CLARK, ELEANOR L., Columbia, Mo.
COLE, WILLIAM H., Professor of Biology, Lake Forest College.
CONKLIN, EDWIN G., Professor of Biology, Princeton University.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COWDRY, EDMUND V., Associate Member, Rockefeller Institute.
COWDRY, NATHANIEL H., 1142 Madison Ave., New York City.
DANCHAKOFF, VERA, Assistant Professor of Anatomy, College of Physicians and Surgeons.
DAWSON, JAMES A., Professor of Biology, Dalhousie University.
DOLLEY, DAVID H., Professor, University of Missouri.
DOLLEY, WILLIAM L., JR., Professor of Biology, Randolph-Macon College.
DONALDSON, HENRY H., Professor of Neurology, Wistar Institute.
DREW, GILMAN A., Assistant Director, Marine Biological Laboratory, Woods Hole, Mass.
GLASER, OTTO, Professor of Biology, Amherst College.
GOLDFARB, A. J., Assistant Professor, College of the City of New York.
GOLDSMITH, WILLIAM M., Professor of Biology, Southwestern College.
GOODRICH, H. B., Associate Professor of Zoölogy, Wesleyan University.
GRAVE, BENJAMIN H., Professor of Zoölogy, Wabash College.
HEILBRUNN, LEWIS V., Assistant Professor of Zoölogy, University of Michigan.
HESS, WALTER N., Professor of Biology, DePauw University.
HIBBARD, HOPE, Associate Professor of Zoölogy, Elmira College.
HOWE, HARRISON E., 2702 36th St., N. W., Washington, D. C.

HUETTNER, ALFRED F., Instructor, Columbia University.
HUMPHREY, RUFUS R., Instructor, Cornell University.
JACOBS, MERKEL H., Assistant Professor of Zoölogy, University of Pennsylvania.
JENNINGS, HERBERT S., Professor of Zoölogy, Johns Hopkins University.
JUST, ERNEST E., Professor of Zoölogy, Howard University.
KNOWER, HENRY MCE., Professor of Anatomy, University of Cincinnati.
LANCEFIELD, DONALD E., Assistant Professor of Zoölogy, Columbia University.
LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri.
LEWIS, WARREN H., Collaborator, Carnegie Institution of Washington.
LEWIS, MARGARET R., Collaborator, Carnegie Institution of Washington.
LILLIE, FRANK R., Chairman, Department of Zoölogy, University of Chicago.
LYNCH, RUTH S., Assistant, Johns Hopkins University.
MCCLUNG, CLARENCE E., Director of Zoölogical Laboratory, University of Pennsylvania.
MCEWEN, ROBERT S., Assistant Professor of Zoölogy, Oberlin College.
MADDOCK, STEPHEN J., Teaching Fellow in Histology, Harvard University Medical School.
MALONE, EDWARD F., Professor of Histology, University of Cincinnati.
MARTIN, EARL A., Assistant Professor, College of the City of New York.
MAST, SAMUEL O., Professor of Zoology, Johns Hopkins University.
METZ, CHARLES W., Member of Staff, Carnegie Institution, Cold Spring Harbor.
MOORE, EMILY L., Research Fellow, Yale University.
MORGAN, THOMAS H., Professor of Experimental Zoölogy, Columbia University.
MORGAN, ANN H., Professor of Zoölogy, Mount Holyoke College.
MORGAN, LILIAN V., 409 West 117th St., New York City.
NOYES, BESSIE, North Carolina College for Women.
PARKER, GEORGE H., Professor of Zoölogy, Harvard University.
PARMENTER, CHARLES L., Instructor, University of Pennsylvania.
PATTEN, WILLIAM, Professor of Biology, Dartmouth College.
PLOUGH, HAROLD H., Associate Professor of Biology, Amherst College.
POTTS, FRANK A., Lecturer in Zoölogy, University of Cambridge.
SCHRADER, FRANZ, Bryn Mawr College.
SHUMWAY, WALDO, Assistant Professor of Biology, Dartmouth College.
SIVICKIS, P. B., University of Chicago.
SMITH, ELIZABETH A., Assistant Professor of Zoölogy, University of Wisconsin.
SPAULDING, EDWARD G., Professor of Philosophy, Princeton University.
SPEIDEL, CARL C., Assistant Professor of Anatomy, University of Virginia.
STARK, MARY B., Professor of Embryology and Histology, New York Hospital Medical College.
STRONG, OLIVER S., Associate Professor of Neurology, Columbia University.
STURTEVANT, ALFRED H., Research Assistant, Carnegie Institution of Washington.
SWETT, FRANCIS H., Instructor in Anatomy, Johns Hopkins University Medical School.
SWINGLE, WILBUR W., Assistant Professor of Biology, Yale University.
SYKES, GEORGE F., Teaching Fellow, Harvard University Medical School.
TENNENT, DAVID H., Professor of Biology, Bryn Mawr College.
TRACY, HENRY C., Professor of Anatomy, University of Kansas.
WEINSTEIN, ALEXANDER, Research Fellow, Columbia University.
WIEMAN, HARRY L., Professor of Zoölogy, University of Cincinnati.
WOODRUFF, LORANDE L., Professor of Biology, Yale University.

WOODWARD, ALVALYN E., Amherst College.
YOUNG, BENJAMIN P., Assistant Professor of Zoölogy, Cornell University.
YOUNG, DONNELL B., Assistant Professor of Biology, Carleton College.

BEGINNING INVESTIGATORS—Zoölogy.

BEAN, RAYMOND J., Instructor in Biology, Western Reserve University.
BENNITT, RUDOLPH, Teaching Fellow, Harvard University.
BRAILEY, MIRIAM E., Assistant in Embryology, Mount Holyoke College.
BROWN, ALICE L., Assistant in Anatomy, Cornell University Medical College.
CHACE, EUNICE E., Instructor, Smith College.
CHASE, RUTH W., Assistant in Zoölogy, University of Wisconsin.
COLE, ELBERT C., Trinity College.
EMERSON, STERLING H., Carnegie Institution, Cold Spring Harbor.
FERRY, RUTH M., Assistant, Carnegie Institution, Cold Spring Harbor.
FRY, HENRY J., Columbia University.
GILSON, ARTHUR S., JR., Research Student, Harvard University.
HAYDEN, MARGARET A., Instructor in Zoölogy, Wellesley College.
HINRICHS, MARIE A., University of Chicago.
HOADLEY, LEIGH, Assistant, University of Chicago.
JOHNSON, H. HERBERT, Laboratory Assistant in Zoölogy, Columbia University.
JOHNSON, GEORGE E., Graduate Student, Harvard University.
LACKEY, JAMES B., Instructor in Zoölogy, Mississippi College.
LOWMAN, EDITH, Assistant Instructor, Yale University.
LOWTHER, FLORENCE DEL., Instructor, Barnard College.
MACBRIDE, LAVINIA G., Graduate Student, University of Michigan.
MACDOUGALL, MARY S., Head of Biology Dept., Agnes Scott College.
MOSES, MILDRED S., Assistant, Carnegie Institution, Cold Spring Harbor.
OVERSTREET, MRS. HARRY A., 802 West 181st St., New York City.
SMITH, CHRISTIANNA, Graduate Fellow, Cornell University.
STURTEVANT, PHOEBE R., Carnegie Institution of Washington.
THARALDSEN, CONRAD E., Assistant Professor of Zoölogy, Northwestern University.
THATCHER, LLOYD E., Instructor in Zoölogy, University of Michigan.
UHLEMEYER, BERTHA, Instructor in Zoölogy, Washington University.
VARIAN, BASIL B., Columbia University.
VICARI, EMILIA, Columbia University.
VISSCHER, J. PAUL, Fellow, Washington University.
WALLACE, EDITH M., Carnegie Institution.
WARREN, HERBERT S., Assistant in Zoölogy, Columbia University.
WILLIAMS, STEPHEN C., Wesleyan University.

INDEPENDENT INVESTIGATORS—Physiology.

BISHOP, GEORGE H., Associate, Washington University Medical College.
BRADLEY, HAROLD C., Professor of Physiological Chemistry, University of Wisconsin.
CLOWES, GEORGE H. A., Research Director, Eli Lilly & Co., Indianapolis, Ind.
COLLETT, MARY E., Instructor in Physiology, University of Buffalo.
COHN, EDWIN J., Assistant Professor of Physiological Chemistry, Harvard University Medical School.
DENIS, WILLY, Associate Professor of Physiological Chemistry, Tulane University.

EDWARDS, DAYTON J., Associate Professor of Physiology, Cornell University Medical College.
GARREY, WALTER E., Professor of Physiology, Tulane University.
GEISER, SAMUEL W., Assistant Professor of Zoölogy, Washington University.
GREISHEIMER, ESTHER M., Instructor in Physiology, University of Minnesota.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HECHT, SELIG, National Research Council Fellow, Harvard University.
HITCHCOCK, DAVID I., Assistant, Rockefeller Institute for Medical Research.
HOPKINS, HOYT S., Assistant Professor of Physiology, Baylor University Medical College.
IRWIN, MARIAN, Research Worker, Radcliffe College.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University.
LILLIE, RALPH S., Biologist, Nela Research Laboratories, Cleveland, Ohio.
LOEB, JACQUES, Head of Division of Experimental Biology, Rockefeller Institute for Medical Research.
LOEB, LEO, Professor of Comparative Pathology, Washington University.
MOORE, ARTHUR R., Professor of Physiology, Rutgers College.
MORGULIS, SERGIUS, Professor of Biochemistry, University of Nebraska, College of Medicine.
POND, SAMUEL E., Biologist, Nela Research Laboratories, Cleveland, Ohio.
RAPPORT, ANNE Y., Associate in Physiology, Bryn Mawr College.
REDFIELD, ALFRED C., Assistant Professor of Physiology, Harvard University Medical School.
SMITH, HOMER W., Eli Lilly & Co., Indianapolis, Ind.
SPAETH, REYNOLD A., Associate in Physiology, School of Public Health, Johns Hopkins University.
SPARROW, CARROLL M., Professor of Physics, University of Virginia.
WARREN, HOWARD C., Professor of Psychology, Princeton University.

BEGINNING INVESTIGATORS—Physiology.

BIERMAN, JESSIE M., Medical Student, University of Chicago.
BRIGHT, ELIZABETH M., Research Assistant in Physiology, Harvard University Medical School.
BURLINGHAM, ROBERT, 156 East 66th St., New York City.
CATTELL, WARE, Garrison, N. Y.
HENDRY, JESSIE L., Technician, Harvard University Medical School.
KLOPP, JOHN W., University of Pennsylvania, School of Medicine.
PAGE, IRVINE H., Chemist, Eli Lilly & Co., Indianapolis, Ind.
SAMPSON, MYRA M., Assistant Professor of Zoölogy, Smith College.
SHEPARD, CHARLES E., Teaching Fellow, University of Minnesota.
STUDEBAKER, MABEL T., Eli Lilly & Co., Indianapolis, Ind.
WALDEN, EDA B., Chemist, Eli Lilly & Co., Indianpaolis, Ind.

INDEPENDENT INVESTIGATORS—Botany.

BROOKS, SUMNER C., Hygienic Laboratory, United States Public Health Service.
BROOKS, MRS. S. C., Hygienic Laboratory, United States Public Health Service.
CLELAND, RALPH E., Assistant Professor of Biology, Goucher College.
EAST, EDWARD M., Professor of Experimental Plant Morphology, Harvard University.

KYLIN, HAROLD, Lund, Sweden.
LEWIS, IVEY F., Professor of Biology, University of Virginia.
LYMAN, GEORGE R., U. S. Department of Agriculture, Washington, D. C.
MOORE, GEORGE T., Director Missouri Botanical Gargen, St. Louis, Mo.
OSTERHOUT, WINTHROP J. V., Professor of Botany, Harvard University.
PHILLIPS, EVERETT F., U. S. Department of Agriculture, Washington, D. C.
RAY, GEORGE B., Instructor in Applied Physiology, Harvard University Medical School.
SCHRAMM, J. R., Professor of Botany, Cornell University.
SNOW, LAETITIA M., Associate Professor of Botany, Wellesley College.
TAYLOR, WILLIAM R., Assistant Professor of Botany, University of Pennsylvania.
WESTON, WILLIAM H., JR., Assistant Professor of Botany, Harvard University.

BEGINNING INVESTIGATORS—Botany.

COOK, SHERBURNE F., Teaching Fellow, Harvard University.
HARRIS, EARL S., Harvard University.
HARVEY, PAUL A., Teaching Fellow, Harvard University.
KEEFE, ANSELM M., Instructor, St. Norbert's College.
LYON, CHARLES J., Instructor in Biology, Dartmouth College
MACINNES, JEAN, Massachusetts Institute of Technology.
MACKAYE, ROBERT K., Student, Harvard University.

STUDENTS.

1922.

BOTANY.

BRISTOL, MARY L., Student, Connecticut College.
EASTON, CHARLOTTE, Head of Biology Dept., Skidmore College.
HESS, FLORENCE G., Student, Cornell University
MCNAIR, MRS. GEORGE T., Chickasha, Okla.
NUGENT, GERTRUDE V., Teacher, East Boston, Mass.
PAGE, HELEN D., Student, University of Chicago.
SMITH, FANNY FERN, Student, Washington University
WOODHEAD, ARTHUR E., Professor of Biology, Western Maryland College.

EMBRYOLOGY.

ANDERSON, PEARL, Assistant in Zoölogy, Vassar College.
BRADLEY, LILLIAN H., Columbia University.
BROWN, LOUISE K., Student, Agnes Scott College.
BUTLER, ELMER G., Instructor in Zoölogy, University of Vermont.
CHAMBERLAIN, WILLIAM H., Columbia University.
CHANG, C., Student, Harvard Medical School.
HALLAUER, EMILY E., Swarthmore College.
HALTER, CLARENCE R., Instructor in Zoölogy, New York University.
HAWKINS, MARY O'NEIL, 1331 Columbine St., Denver, Colorado.
HULPIEU, HAROLD R., Student, Southwestern College.
LEVY, JOSEPH, Student, Johns Hopkins University.
LILLIE, MARGARET H., Student, Mount Holyoke College.

LUCAS, ALFRED M., Student, Wabash College.
MCCAA, FANNY, Instructor, Agnes Scott College.
METCALF, RACHEL V., Instructor in Zoölogy, Mount Holyoke College.
MILLER, HARRY M., JR., Fellow in Zoölogy, University of Illinois.
MILLER, FRANKLIN R., Student, Illinois Wesleyan University
PIERSON, CHARLES J., Professor of Zoölogy, University of Maryland.
ROBINSON, OWEN L., Student, De Pauw University.
SHAVER, JESSE M., Assistant Professor of Biology, George Peabody College.
STEEN, EDWIN B., Assistant in Zoölogy, Wabash College.
STEVENS, EDITH, West Virginia University.
STEWART, COLIN C., JR., Student, Dartmouth College.
STEWART, DOROTHY R., Assistant, Washington University.
TOWLER, VIOLA, Shorter College.
WASSUM, EVA E., Student, Agnes Scott College.
WICKS, NINA A., Student, Knox College.
WILCOX, GLADYS, University of Delaware.

PHYSIOLOGY.

BROWN, MADELAINE R., Social Worker, State Hospital for Mental Diseases, Howard, R. I.
BROWN, MARY J., Associate Professor, Transylvania College.
COVENTRY, FRANCES A., Assistant, Biology Dept., Goucher College.
DICKINSON, PORTER S., Student, Harvard Medical School.
DIMICK, G. PRISCILLA, Smith College.
DRAKE, DOROTHY, Assistant in Physiology, Mount Holyoke College.
FLEXNER, LOUIS B., Student, University of Chicago.
GILMAN, CHARLOTTE W., Instructor in Zoölogy, Vassar College.
HARTMAN, ARTHUR M., 1414 Girard St., N. W., Washington, D. C.
ISZARD, MIRIAM S., Instructor in Bacteriology, University of Pennsylvania.
LANDIS, EUGENE M., Student, University of Pennsylvania.
LEWTON, LUCY O., Barnard College.
LINDSAY, BLANCHE, Assistant in Zoolögy and Physiology, Wellesley College.
MORRISON, THOMAS F., Student, Princeton University.
PATCH, ESTHER M., Teacher, Stoneham, Mass.
REYNOLDS, PHILIP A., Boston University School of Medicine.
SLOAN, LOUISE L., Student, Bryn Mawr College.
WHITE, E. GRACE, Professor of Biology, Shorter College.
WOOLLARD, HERBERT H., Lecturer in Anatomy, University College, London.

PROTOZOÖLOGY.

BAKER, W. B., Assistant Professor of Biology, Emory University.
BOX, CORA M., Assistant Professor, University of Cincinnati.
BOYD, GEORGE H., Instructor in Biology, Emory University.
COUTANT, MARY W., Instructor in Botany, Barnard College.
EMMART, EMILY W., 817 N. Fremont Ave., Baltimore, Md.
GRAVES, ISABELLE A., Assistant Bacteriologist, City Laboratory, New Haven, Conn.
NOLAND, LOWELL E., Instructor, Zoology Dept., University of Wisconsin.
SCHRADER, SALLY H., Bryn Mawr, Pa.

TAO, WEI SUN, Student, Columbia University.
TILDEN, EVELYN B., Rockefeller Institute.
UNGER, W. BYERS, Instructor in Biology, Lafayette College.
WALTERS, MARY J., Instructor, Goucher College.

ZOÖLOGY.

ANDERSON, ETHEL L., University of Kentucky.
ANDERSON, HOPE E., Student, Mount Holyoke, College.
BAMBER, MAURINE, Student, Knox College.
BATON, GERTRUDE M., Assistant Instructor in Biology, Carnegie Institute of Technology.
BROWN, BERNICE D., Student, Oberlin College.
BUEHLER, EUGENE O., Student, Wabash College.
BURWELL, MARGARET S., Student, Sweet Briar College.
CAMPBELL, EVA G., Instructor, North Carolina College for Women.
COOPER, DRURY W., JR., Student, Rutgers College.
COPENHAVER, WILFRED M., Assistant in Biology, Yale University.
DENBY, EDWIN O., Student, Harvard College.
DIXON, PERRINE C., Student, Sophie Newcomb College.
DOOLEY, PARKER, Student, Illinois Wesleyan University.
FAW, HELEN A., Student, Agnes Scott College.
FEDERIGHI, HENRY, Student, Rutgers College.
FULLER, ANDREW B., Student, University of Pennsylvania.
GORHAM, GRACE V., Undergraduate student, Mount Holyoke College.
GRANT, JEAN F., Student, Sweet Briar College.
GRAY, IRVING E., Instructor, DePauw University.
GRAY, NINA E., Student, DePauw University.
HAGEDON, EDITH L., Student, DePauw University.
HALL, FRANK G., Assistant in Zoölogy, University of Wisconsin.
HOPKINS, AUBREY E., College of William and Mary.
HOUGHTON, DOROTHY, Student, Barnard College.
HUBER, ERNST, Associate in Anatomy, Johns Hopkins Medical School.
HUNSICKER, MARY G., Student, University of Pennsylvania.
JONES, MYRNA F., Student, Doane College.
KENNAN, ADA B., Assistant in Zoölogy, University of Michigan.
KUBICEK, HELEN M., Student, Doane College.
LEWIS, MARION F., Student, Mount Holyoke College.
LUCE, ROBERT H., Carleton College.
MAGUIRE, GERTRUDE A., Student, Hunter College.
MASON, KARL E., Assistant in Zoölogy, Yale University.
MCNAIR, GEORGE T., Professor of Zoölogy, Oklahoma College for Women.
MIEHLING, RUTH B., Student, Hunter College.
MILLER, GLENN O., Student, Southwestern College.
MORGAN, RUBY M., Student, Oberlin College.
NICKELL, FAITH E., Washington University.
NORTON, HERMON, Student, Wesleyan University.
NYI, TSUNG-TSONG, Student, Smith College.
PAYNE, MARY G., Student, Butler College.
PERRY, LYDIA S., Student, Oberlin College.
PRITCHARD, MAYBELLE, Student, Radcliffe College.

REED, LUCILE L., Student, Sophie Newcomb College.
RILEY, PHILIP L., Student, Massachusetts Institute of Technology.
ROSE, BERTHA E., Assistant in Zoölogy, University of Wisconsin.
RUSTIA, CONSTANCIO P., Graduate Student, University of Chicago.
SAMPSON, HOWARD J., Massachusetts Agricultural College.
SCOTT, GORDON H., Assistant, Johns Hopkins University.
SOISSON, MARY C., Goucher College.
STEVENS, DOROTHY H., Student, Connecticut College.
STIFFLER, ETHEL G., Student, Goucher College.
STRAUSS, MAURICE B., Student, Amherst College.
SUMWALT, MARGARET, Student, Goucher College.
TAFT, CHARLES H., JR., Instructor in Biology, Tufts College.
TAYLOR. ELIZABETH R., University of Delaware.
THEOCHARIDES, ELECTRA, Assistant in Biology, Constantinople College.
TINGLEY, MARY A., Instructor, Shorter College.
VAN HORN, AMEY D., Instructor in Biology, Alfred University.

3. TABULAR VIEW OF ATTENDANCE.

	1918	1919	1920	1921	1922
INVESTIGATORS—Total	93	134	136	172	182
Independent:					
Zoölogy	51	68	69	79	87
Physiology	16	24	22	26	28
Botany	5	7	7	13	15
Under Instruction:					
Zoölogy	16	21	29	34	34
Physiology	3	10	7	11	11
Botany	2	4	2	9	7
STUDENTS—Total	69	128	120	120	126
Zoölogy	41	55	56	53	59
Protozoölogy	—	15	15	11	12
Embryology	12	33	26	28	28
Physiology	10	17	15	18	19
Botany	6	8	8	10	8
TOTAL ATTENDANCE	162	262	256	292	308
INSTITUTIONS REPRESENTED—					
Total	72	88	86	95	104
By investigators	49	61	55	67	71
By students	38	62	57	53	68
SCHOOLS AND ACADEMIES REPRESENTED.					
By investigators	—	—	1	—	—
By students	—	4	7	—	—

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS IN 1922.

Amherst College.
Barnard College.
Bowdoin College.
Bryn Mawr College.
Butler College.
Carnegie Institution of Washington.
Carnegie Institution, Cold Spring Harbor.
Carnegie Institute of Technology.
College of Physicians and Surgeons.
Columbia University.
Connecticut College.
Cornell University.
Cornell University Medical College.
Dartmouth College.
DePauw University.
Doane College.
Eli Lilly & Co.
Goucher College.
Harvard University.
Harvard University Medical School.
Hunter College.
Illinois Wesleyan University.
Johns Hopkins University.
Johns Hopkins University Medical School.
Knox College.
Lake Forest College.
Massachusetts Institute of Technology.
Mount Holyoke College.
Nela Research Laboratories.
North Carolina College for Women.
Oberlin College.
Princeton University.
Radcliffe College.
Rockefeller Foundation.
Rockefeller Institute for Medical Research.
Rutgers College.
Sophie Newcomb College.
Southwestern College.
Sweet Briar College.
Tufts College.
U. S. Veterans Bureau.
University of Chicago.
University of Cincinnati.
University of Delaware.
University of Illinois.
University of Kansas.
University of Maryland.
University of Michigan.
University of Minnesota.
University of Pennsylvania.
University of the Philippines.
University of Vermont.
University of Wisconsin.
Vassar College.
Wabash College.
Washington University.
Wesleyan University.
Wellesley College.
Western Reserve University.
Wistar Institute of Anatomy and Biology.
Yale University.

Scholarship Tables.

The Lucretia Crocker Scholarships for Teachers in Boston, Since 1888.

Scholarship of $100.00, Supported by a Friend of the Laboratory, Since 1898.

The New London Branch of the American Association of University Women, Since 1920.

5. EVENING LECTURES, 1922.

Friday, June 30,
DR. SELIG HECHT. . . . . "The Visibility of the Spectrum."

Friday, July 7,
DR. H. S. JENNINGS. . . . . "A Critical Study of the So-called Linear Theory of Crossing-Over in Inheritance."

Tuesday, July 11,
DR. D. H. DOLLEY. . . . . "Specific Functions and Specific Irritability."

Friday, July 14,
DR. GEORGE SARTON. . . . . "The History of Science."

Tuesday, July 18,
DR. E. E. JUST. . . . . "Certain Effects of Hypertonic Sea-water in Activating *Arbacia* Eggs."

Friday, July 21,
MR. WM. LYMAN UNDERWOOD. . "Wild Brother."

Monday, July 24,
DR. J. A. DETLEFSEN. . . . . "Experiments Regarding the Inheritance of the Effects of Rotation in Rats."

Tuesday, July 25,
DR. C. B. BRIDGES. . . . . "Chromosomes and Inheritance in *Drosophila melanogaster*."

Friday, July 28,
DR. G. H. PARKER. . . . . "Physiology of Actinian Muscle."

Monday, July 31,
DR. W. H. WESTON. . . . . "Following a Fungus through the Philippines."

Tuesday, August 1,
DR. W. H. LEWIS. . . . . "Tissue Culture."

Friday, August 4,
DR. J. R. SCHRAMM. . . . . "Some Thoughts on Abstracting and Indexing Biological Literature."

Tuesday, August 8,
DR. W. D. BANCROFT. . . . . "Structural Colors in Feathers."

6. MEMBERS OF THE CORPORATION.

1. Life Members.

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Carey, Mr. Arthur Astor, Fayerweather St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
Marrs, Mrs. Laura Norcross, 9 Commonwealth Ave., Boston, Mass.
Mason, Miss E. F., 1 Walnut St., Boston, Mass.
Mason, Miss Ida M., 1 Walnut St., Boston, Mass.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Columbia University, New York City, N. Y.

MORGAN, MRS. T. H., New York City, N. Y.
NOYES, MISS EVA J.
NUNN, MR. LUCIAN L., Telluride, Colo.
OSBORN, PROF. HENRY F., American Museum of Natural History, New York City, N. Y.
PHILLIPS, DR. JOHN C., Windy Knob, Wenham, Mass.
PHILLIPS, MRS. JOHN C., Windy Knob, Wenham, Mass.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pa.
PULSIFER, MR. W. H., Newton Center, Mass.
ROGERS, MISS A. P., 5 Joy St., Boston, Mass.
SEARS, DR. HENRY F., 86 Beacon St., Boston, Mass.
SHEDD, MR. E. A.
THORNDIKE, DR. EDWARD L., Teachers College, Columbia University, New York City, N. Y.
TRELEASE, PROF. WILLIAM, University of Illinois, Urbana, Ill.
WARE, MISS MARY L., 41 Brimmer St., Boston, Mass.
WHITNEY, MR. HENRY M., Brookline, Mass.
WILCOX, MISS MARY A., Wellesley College, Wellesley, Mass.
WILLIAMS, MRS. ANNA P., 505 Beacon St., Boston, Mass.
WILSON, DR. E. B., Columbia University, New York City, N. Y.
WILSON, PROF. W. P., Commercial Museum, Philadelphia, Pa.

2. REGULAR MEMBERS, AUGUST, 1922.

ADAMS, MISS A. E., Mount Holyoke College, South Hadley, Mass.
ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.
ADOLPH, DR. EDWARD F., University of Pittsburgh, Pittsburgh, Pa.
AGERSBORG, DR. H. P. K., University of Nebraska, Lincoln, Neb.
ALLEE, DR. W. C., University of Chicago, Chicago, Ill.
ALLEN, PROF. EZRA, Ursinus College, Collegeville, Pa.
ALLYN, MISS HARRIET M., Hackett Medical College, Canton, China.
ALTENBURG, DR. EDGAR, Rice Institute, Houston, Texas.

ANDERSON, DR. E. G., College of the City of New York, New York City.

ATTERBURY, MRS. RUTH R., College of Physicians and Surgeons, New York City, N. Y.

BAITSELL, DR. GEORGE A., Yale University, New Haven, Conn.

BAKER, MRS. L. D., 123 Chiswick Road, Boston, Mass.

BAKER, DR. E. H., 5729 Kimbark Ave., Chicago, Ill.

BALDWIN, DR. F. M., Iowa State College, Ames, Iowa.

BANCROFT, PROF. F. W., Aloha Farm, Concord, Calif.

BASCOM, DR. K. F., Allegheny College, Meadville, Pa.

BECKWITH, DR. CORA J., Vassar College, Poughkeepsie, N. Y.

BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, La.

BIGELOW, PROF. M. A., Teachers College, Columbia University, New York City, N. Y.

BIGELOW, PROF. R. P., Massachusetts Institute of Technology, Cambridge, Mass.

BINFORD, PROF. RAYMOND, Guilford College, Guliford College, N. C.

BORING, DR. ALICE M., Wellesley College, Wellesley, Mass.

BOX, MISS CORA MAY, University of Cincinnati, Cincinnati, Ohio.

BOWEN, DR. ROBERT H., Columbia University, New York City, N. Y.

BRADLEY, PROF. HAROLD C., University of Wisconsin, Madison, Wis.

BRAILEY, MISS MIRIAM E., Mount Holyoke College, South Hadley, Mass.

BRIDGES, DR. CALVIN B., Columbia University, New York City, N. Y.

BROOKS, DR. S. C., U. S. Public Health Service, Washington, D. C.

BRUMFIEL, DR. DANIEL M., University of Iowa, Iowa City, Iowa.

BUCKINGHAM, MISS EDITH N., 342 Marlboro St., Boston, Mass.

BUDINGTON, PROF. R. A., Oberlin College, Oberlin, Ohio.

BUMPUS, DR. H. C., Brown University, Providence, R. I.

BYRNES, DR. ESTHER F., 193 Jefferson Ave., Brooklyn, N. Y.

CALKINS, PROF. GARY N., Columbia University, New York City, N. Y.

CALVERT, PROF. PHILIP P., University of Pennsylvania, Philadelphia, Pa.

CARLSON, PROF. A. J., University of Chicago, Chicago, Ill.

CAROTHERS, DR. ELEANOR E., University of Pennsylvania, Philadelphia, Pa.

CARPENTER, PROF. FREDERIC W., Trinity College, Hartford, Conn.

CARROLL, PROF. MITCHEL, Franklin and Marshall College, Lancaster, Pa.

CARVER, PROF. GAIL L., West Lake, Ga.

CASEY, COLONEL THOMAS L., Washington, D. C.

CASTEEL, DR. D. B., University of Texas, Austin, Texas.

CATTELL, PROF. J. MCKEEN, Garrison-on-Hudson, N. Y.

CATTELL, MR. MCKEEN, Harvard Medical School, Boston, Mass.

CHAMBERS, DR. ROBERT, Cornell University Medical College, New York City, N. Y.

CHARLTON, DR. HARRY H., University of Missouri, Columbia, Mo.

CHIDESTER, PROF. F. E., West Virginia University, Morgantown, W. Va.

CHILD, PROF. C. M., University of Chicago, Chicago, Ill.

CLAPP, PROF. CORNELIA M., Mount Holyoke College, South Hadley, Mass.

CLARK, PROF. E. R., University of Georgia, Augusta, Ga.

CLOWES, DR. G. H. A., Eli Lilly & Co., Indianapolis, Ind.

COE, PROF. W. R., Yale University, New Haven, Conn.

COHN, DR. EDWIN J., 25 Follen St., Cambridge, Mass.

COLE, DR. LEON J., College of Agriculture, Madison, Wis.

COLLETT, DR. MARY E., Ashland, Mass.

COLTON, PROF. H. S., Ardmore, Pa.

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COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Maine.

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COWDRY, DR. E. V., Rockefeller Institute, New York City, N. Y.

CRAMPTON, PROF. H. E., Barnard College, Columbia University, New York City, N. Y.

CRANE, MRS. C. R., Woods Hole, Mass.

CURTIS, PROF. W. C., University of Missouri, Columbia, Mo.

CURTIS, DR. MAYNIE R., Crocker Laboratory, Columbia University, New York City, N. Y.

DANCHAKOFF, DR. VERA, College of Physicians and Surgeons, New York City, N. Y.

DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Va.

DAVIS, PROF. BRADLEY M., University of Michigan, Ann Arbor, Mich.

DAVIS, DR. ALICE R., Harvard Medical School, Boston, Mass.

DAWSON, DR. J. A., Dalhousie University, Halifax, Nova Scotia.

DEDERER, DR. PAULINE H., Connecticut College, New London, Conn.

DEXTER, DR. J. S., Northwestern College, Naperville, Ill.

DODDS, PROF. G. S., Medical School, University of West Virginia, Morgantown, W. Va.

DONALDSON, PROF. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

DONALDSON, DR. JOHN C., University of Pittsburgh, School of Medicine, Pittsburgh, Pa.

DREW, PROF. GILMAN A., Marine Biological Laboratory, Woods Hole, Mass.

DUNGAY, DR. NEIL S., Carleton College, Northfield, Minn.

DUNN, DR. ELIZABETH H., Woods Hole, Mass.

EDWARDS, DR. D. J., Cornell University Medical College, New York City, N. Y.

EIGENMANN, PROF. C. H., University of Indiana, Bloomington, Ind.

ELLIS, DR. F. W., Monson, Mass.

FARNUM, MISS LOUISE W., Peking, China.

FIELD, MISS HAZEL E., Sophie Newcomb College, New Orleans, La.

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FRY, MR. HENRY J., Columbia University, New York City, N. Y.

GAGE, PROF. S. H., Cornell University, Ithaca, N. Y.

GARREY, PROF. W. E., Tulane University, Richardson Memorial, New Orleans, La.

GATES, PROF. R. RUGGLES, University of London, London, England.

GLASER, PROF. O. C., Amherst College, Amherst, Mass.

GLASER, PROF. R. W., Rockefeller Institute for Medical Research, Princeton, N. J.

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GUYER, PROF. M. F., University of Wisconsin, Madison, Wis.

HANCE, DR. ROBERT T., North Dakota Agricultural College, Fargo, N. D.

HARGITT, PROF. C. W., Syracuse University, Syracuse, N. Y.

HARGITT, PROF. GEORGE T., Syracuse University, Syracuse, N. Y.

HARMAN, DR. MARY T., Kansas State Agricultural College, Manhattan, Kansas.

HARPER, PROF. R. A., Columbia University, New York City, N. Y.

HARRISON, PROF. ROSS G., Yale University, New Haven, Conn.

HARVEY, PROF. E. N., Princeton University, Princeton, N. J.

HARVEY, MRS. E. N., Princeton, N. J.

HAYDEN, MISS MARGARET A., Wellesley College, Wellesley, Mass.

HEATH, PROF. HAROLD, Palo Alto, Calif.

HEGNER, PROF. R. W., Johns Hopkins University, Baltimore, Md.

HEILBRUNN, DR. L. V., University of Michigan, Ann Arbor, Mich.

HESS, PROF. WALTER N., DePauw University, Greencastle, Ind.

HINRICHS, MISS MARIE A., Nela Research Laboratory, Cleveland, Ohio.

HOADLEY, DR. LEIGH, University of Chicago, Chicago, Ill.

HOGUE, DR. MARY J., North Carolina College for Women, Greensboro, N. C.

HOLMES, PROF. S. J., University of California, Berkeley, Calif.

HOPKINS, DR. HOYT S., Baylor University, Dallas, Texas.

HOSKINS, MRS. ELMER R., University of Arkansas Medical School, Little Rock, Ark.

HOWE, DR. H. E., National Research Council, Washington, D. C.

HOWLAND, PROF. RUTH B., Sweet Briar College, Sweet Briar, Va.

HOYT, DR. WILLIAM D., Washington and Lee University, Lexington, Va.

HUMPHREY, MR. R. R., Cornell University, Ithaca, N. Y.

HYMAN, DR. LIBBIE H., University of Chicago, Chicago, Ill.

JACKSON, PROF. C. M., University of Minnesota, Minneapolis, Minn.

JACOBS, PROF. MERKEL H., University of Pennsylvania, Philadelphia, Pa.

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JONES, PROF. L. R., National Research Council, Washington, D. C.

JORDAN, PROF. H. E., University of Virginia, Charlottesville, Va.

JUST, PROF. E. E., Howard University, Washington, D. C.

KEEFE, REV. ANSELM M., St. Norbert's College, West Depere, Wis.

KENNEDY, DR. HARRIS, Readville, Mass.
KINDRED, DR. J. E., Western Reserve University, Cleveland, Ohio.
KING, DR. HELEN D., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
KING, DR. ROBERT L., University of Pennsylvania, Philadelphia, Pa.
KINGSBURY, PROF. B. F., Cornell University, Ithaca, N. Y.
KINGSLEY, PROF. J. S., University of Illinois, Urbana, Ill.
KIRKHAM, DR. W. B., Springfield College, Springfield, Mass.
KNOWER, PROF. H. McE., University of Cinncinati, Cincinnati, Ohio.
KNOWLTON, PROF. F. P., Syracuse University, Syracuse, N. Y.
KOSTIR, DR. W. J., Ohio State University, Columbus, Ohio.
KRIBS, DR. HERBERT, University of Pennsylvania, Philadelphia, Pa.
KUYK, DR. MARGARET P., Richmond, Va.
LANCEFIELD, DR. D. E., Columbia University, New York City, N. Y.
LANGE, DR. MATHILDE M., Wheaton College, Norton, Mass.
LEE, PROF. F. S., 437 West 59th St., New York City, N. Y.
LEWIS, PROF. I. F., University of Virginia, Charlottesville, Va.
LEWIS, PROF. W. H., Johns Hopkins University, Baltimore, Md.
LILLIE, PROF. FRANK R., University of Chicago, Chicago, Ill.
LILLIE, PROF. R. S., Nela Research Laboratory, Nela Park, Cleveland, Ohio.
LINTON, PROF. EDWIN, 1104 Milledge Road, Augusta, Ga.
LOEB, PROF. JACQUES, Rockefeller Institute for Medical Research, New York City, N. Y.
LOEB, PROF. LEO, Washington University Medical School, St. Louis, Mo.
LOEB, MRS. LEO, St. Louis, Mo.
LOWTHER, MRS. FLORENCE DeL., Barnard College, Columbia University, New York City, N. Y.
LUND, DR. E. J., University of Minnesota, Minneapolis, Minn.
LUSCOMBE, MR. W. O., Woods Hole, Mass.
LYMAN, PROF. GEORGE R., Plant Disease Survey, Department of Agriculture, Washington, D. C.

LYNCH, MISS CLARA J., Rockefeller Institute, New York City, N. Y.

LYON, PROF. E. P., University of Minnesota, Minneapolis, Minn.

MACCALLUM, DR. G. A., 925 St. Paul St., Baltimore, Md.

MACDOUGALL, MISS MARY S., Agnes Scott College, Decatur, Ga.

MCCLUNG, PROF. C. E., University of Pennsylvania, Philadelphia, Pa.

MCGEE, DR. A. N., Woods Hole, Mass.

MCGILL, DR. CAROLINE, Murray Hospital, Butte, Montana.

MCGREGOR, DR. J. H., Columbia University, New York City, N. Y.

MCINDOO, DR. N. E., Bureau of Entomology, Washington, D. C.

MCMURRICH, PROF. J. P., University of Toronto, Toronto, Canada.

MACKLIN, DR. CHARLES C., School of Medicine, Western University, London, Canada.

MALONE, PROF. E. F., University of Cincinnati, Cincinnati, Ohio.

MARTIN, MISS BERTHA E., Lindenwood College, St. Charles, Mo.

MAST, PROF. S. O., Johns Hopkins University, Baltimore, Md.

MATHEWS, PROF. A. P., University of Cincinnati, Cincinnati, Ohio.

MATSUI, PROF. K., Imperial College of Agriculture and Dendrology, Morioka, Japan.

MAVOR, PROF. JAMES W., Union College, Schenectady, N. Y.

MEIGS, DR. E. B., Dairy Division Experiment Station, Beltsville, Md.

MEIGS, MRS. E. B., 1445 Rhode Island Ave., Washington, D. C.

MEDES, DR. GRACE, Vassar College, Poughkeepsie, N. Y.

METCALF, PROF. M. M., 128 Forest St., Oberlin, Ohio.

METZ, PROF. CHARLES W., Carnegie Institution of Washington, Cold Spring Harbor, Long Island.

MINER, DR. ROY W., American Museum of Natural History, New York City, N. Y.

MITCHELL, DR. PHILIP H., Brown University, Providence, R. I.

MOORE, PROF. GEORGE T., Missouri Botanical Garden, St. Louis, Mo.

MOORE, DR. CARL R., University of Chicago, Chicago, Ill.
MOORE, PROF. J. PERCY, University of Pennsylvania, Philadelphia, Pa.
MOORE, DR. A. R., Rutgers College, New Brunswick, N. J.
MORGAN, PROF. H. A., Agricultural Experiment Station, Knoxville, Tenn.
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SEX-DIFFERENTIATION IN THE VIVIPAROUS TELEOST *XIPHOPHORUS HELLERI* HECKEL.

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I. INTRODUCTION.

In the fall of 1920, Dr. A. W. Bellamy, who is carrying on breeding experiments with several viviparous teleosts in this laboratory, obtained two *Xiphophorus helleri* from F. E. Boehn, a member of the Chicago Aquarium Club, who claimed that these fish had been subject to sex-inversion. According to this report he had three unusually large specimens—two females and one male—which he kept in a breeding tank and from which he raised more than five hundred young. When the females were about

three years old they ceased producing young and, during the course of several weeks, took on the secondary sex characters of males.

When, a few days later, these specimens came into the writer's possession, both were decidedly female as to shape and size of body and decidedly male as to development of anal and caudal fins, which were far advanced toward the male condition yet much belated. Cytological preparations of the entire reproductive system of both fishes disclosed that ripe sperm was in all parts of the duct and gonad, but that the latter was juvenile in comparison to the size and age of the fish.

Suggestive as the case was, the data obtained were far too meager to decide either for or against the reputed transformation. That peculiarities do occur in the sex conditions of these teleosts has been noted in this laboratory as well as by many fish fanciers. The extent and meaning of these peculiarities became the aim of this work. It was thought necessary to approach this problem from three different angles:

1. A detailed study of sex-differentiation in males and females.
2. Isolation of a large number of females for sex observation.
3. An attempt to control sex experimentally.

Only the first part is considered in the present paper. The last two are still in progress and will be the subject of a future publication.

II. Material and Methods.

No attempt was made to study embryonic development during gestation, except for a few later stages, which will be described further on. Development during gestation seems to present some interesting features which will be further investigated as soon as the opportunity presents itself. My material includes fishes from birth to adult stage. To secure all possible stages in sex-differentiation, a large number of fishes were necessary; not less than 400 specimens were studied, of which 300 were sectioned for cytological study.

As to fixatives, Allen's, Bouin's, Child's, Flemming's strong, Smith's, and Zenker's fluids have been used, Bouin's giving constant and satisfactory results. For general stains Heidenhain's hæmotoxylin and alum-cochineal counterstained with orange G

were used successfully. For special stains Mallory's triple stain and Harris' hæmotoyxlin were used. All material except adult ovaries were sectioned in paraffin, the latter in celloidin. Very young fishes were sectioned as a whole, in medium-sized fishes the entire viscera were sectioned, and in the mature fishes the gonads and ducts only were preserved for sectioning. For general study sections were cut 6 μ in thickness.

The body form of the female is strikingly different from that of the male, which fact can be used to advantage in following the development of the species. To express it in numbers, the length of the fish is divided by its greatest depth. The resulting ratio is known as the form index and can be obtained in two ways, by using either the total length or the body length of the fish as the numerator. The former method is less subject to error in the case of *Xiphophorus helleri* and will be used exclusively in this work.

As the anal fin undergoes a marked post embryonal change in the males in which the third ray increases immensely in diameter, while the fourth and fifth rays, although participating in the transformation, suffer very little, if any, increase in diameter, a ratio of the third by the fourth ray suggests itself. This ratio may be called the fin ratio.

It gives me great pleasure to acknowledge my indebtedness to Prof. F. R. Lillie and Dr. A. W. Bellamy for numerous suggestions and criticisms during the progress of this work. I also wish to express my appreciation for the most painstaking services rendered by our artist, Mr. K. Toda, our technician, Miss D. Brockett, and our librarian, Miss E. L. Dickinson.

III. The Indifferent Stage.

At the time the young fishes are born they measure on the average 8 mm. in total length, while sex is unmistakably established at the length of 10 mm. This means that the indifferent stage of sex development is almost entirely pre-natal. The form index is 6.26 and the fin ratio is 1.0 at birth. The gonads, although small, are distinctly set off from the surrounding tissues and lie one on each side of the body cavity, immediately below the air bladder, suspended in a peritoneal sac. The peritoneal sac, which becomes the permanent wall of the gonad, is a protruded portion of the

lining of the body cavity. The gonad inside of this sac consists of two kinds of cells:

1. Primordial germ cells.

2. Very much smaller cells with elongated nuclei, which can not be distinguished from the peritoneal cells and which tend to surround the germ cells to form follicles (Fig. 1).

The primordial germ cells are unmistakable. They are the largest cells in the body of the fish and measure on the average, cell 14.4 μ, nucleus 8.4 μ, in diameter. The nucleus takes a lighter stain than the cytoplasma and possesses one more or less distinct nucleolus. The chromatin occurs most commonly in strands or loops immediately beneath the nuclear membrane. The germ cells may be single or in nests of two or more cells.

As stated above, the pre-natal developmental changes have not been studied systematically. Several stages, however, are on hand. The gonads assume the bilateral position at about 3.2 mm. in total length. Before that the germ cells are in a single mass placed medially in the body cavity. The peritoneum already surrounds the gonad more or less loosely and here the mingling of the peritoneal cells with the germ cells is very evident (Fig. 3). As these rather small insignificant cells, mingling with the primordial germ cells, play by no means an insignificant rôle in later differentiation, I have been particularly anxious to trace their origin, and must conclude that no criterion whatever separates them from peritoneal cells. This view is consonant with that of McLeod (1881), Jungerson (1889), and Eigenmann (1897). The primordial germ cells of the 3.2 mm. stage are very conspicuous structures. The cell is 11.8 μ and the nucleus is 6.4 μ in diameter. The nucleus may be lobulated and appear as if more than one nucleus was present in a cell (Fig. 3). The cytoplasm is yolk laden. In short, the conditions of these cells indicate an early segregation.

IV. Early Sex-differentiation of the Female.

The earliest stage of the ovary shows very little difference from the indifferent gonad. In the early development of the ovary the primordial germ cells grow in size and form follicles. At the time these follicles have reached approximately the medium size they degenerate and are absorbed. This process is referred to as retro-

gression. Definitive germ cells originate from peritoneal derivatives later and differentiate into functional follicles of the adult ovary. All young females undergo retrogression, of which three classes may be distinguished. In some, hereafter distinguished as class 1, formation of definitive follicles begins before retrogression is well advanced. These comprise about 50 per cent. of all young females (see Table IV., p. 68). In others (class 2) no formation of definitive follicles precedes complete degeneration of primordial follicles, but indifferent germ cells are seen in the epithelium before degeneration is complete. In others, again (class 3), retrogression of primordial germ cells is complete before any germ cells appear in the epithelium. In the first class there is no doubt that development into functional females occurs; the later history of the other two classes is considered beyond. The ovarian cavity and the oviduct are formed comparatively early in the development of the ovary.

1. *Early Normal Ovary.*

The total length of the young female varies from 9.3 to 16.9 mm. The average form index is 5.02 and the fin ratio is 1.13.

The indifferent gonad passes into the ovary very gradually. Paired and considerably apart at the beginning, the two ovaries approach each other until they meet medially and fuse into one gonad which is the normal condition of the adult ovary. The external contour of the gonad is very regular and its component cells, the primordial germ cells and the peritoneal cells, are evenly distributed (Fig. 2). The gonad gradually increases in size owing to the multiplication of its component cells, although no mitotic figures have been discovered. This is true of all the material on hand.

At the time the two gonads have approached each other the picture has changed considerably. The primordial germ cells increase in size and become completely invested by peritoneal cells to form a follicle of one layer of cells thick. The growth of the germ cells is not simultaneous, some grow faster than others, but all the germ cells present in the gonad are subject to a transformation to young ova at this stage or the early phase of the next stage. Blood vessels which are present in the mesentery enter the young ovary in the form of capillaries.

The shape of the approaching gonads changes. Instead of being broader laterally they now deepen dorso-ventrally. Fusion of the two gonads take place anterio-posteriorly, not along the entire surface of contact, but on the dorsal and ventral margins only. The space between becomes the ovarian cavity (Figs. 4–5). It will be recalled that the external lining of the gonad is of peritoneal origin, which means that the epithelium of the ovarian cavity is of peritoneal origin. At first the ovarian cavity is a narrow slit with its larger axis running dorso-ventrally; very soon, however, this picture changes to a cross-shaped lumen produced by the invagination of the epithelium laterally. This form of the lumen persists for a considerable time in the young ovary, but ultimately becomes very much modified until in the adult ovary no trace of the original form remains (Fig. 27). That portion of the mesentery which comes to lie between the fusing gonads becomes absorbed. The dorsal portion, or mesovarium, attaches the ovary to the body wall dorsally, while the ventral portion connects the gonad with the rectum ventrally.

The formation of the oviduct is begun at the time the two gonads are in close proximity. By the time the gonads are completely fused the duct tissue extends from ovary to urogenital sinus with complete lumen at anterior and posterior ends, but none or only a beginning centrally. In fact, the lumen of the oviduct appears first in its posterior terminal, and only later, at the anterior, end. This, of course, would indicate that the duct formation proceeds from two primordia, one anterior and the other posterior, which is the case in *Xiphophorus helleri*. The anterior primordium is the posterior end of the ovary and the posterior is the lining of the body cavity at its most posterior end in the region of the urogenital sinus. Before there is any indication of duct formation anteriorly, the posterior primordium is actively proliferating cells which at first form a solid cord along the median portion of the peritoneal lining of the body cavity on the dorsal edge of the mesentery. This cord extends anteriorly and at the time a similar strand of cells is formed from the anterior primordium a lumen has appeared in the former posteriorly (Figs. 10–11). No liquefaction of cells has been noticed in the formation of the lumen.

The anterior cord of cells is a direct extension of the posterior

ventral portion of the ovary. As that part of the ovary has not as yet fused dorsally, the extending cord is V-shaped with the apex ventrally (Fig. 6). This portion of the duct grows posteriorly on the dorsal edge of the mesentery until it reaches and fuses with its posterior member. The mesovarium thickens and flattens above the posterior portion of the ovary and finally comes in contact with the open end of the V to fuse and thus form the lumen in the oviduct from the anterior primordium (Figs. 6–8).

That the duct starts to grow from two sources was first noticed in an abnormality in which the two growing tips overlapped with the urinary bladder between. In normal development the duct forms ventral to the urinary system, but in the case in question the anterior part of the duct had pushed above the urinary bladder, while that from the posterior end was in the normal path. The overlapping was for a considerable distance. This stimulated closer study, and it was found that at the time when cords are formed at both ends no traces of such exist centrally (Fig. 14), nothing but a single layer of peritoneal lining. Further it was ascertained that by far the larger part of the duct comes from the posterior source.

In the primordial germ cells of the indifferent stage there is only one nucleolus with irregular, generally spherical contour. It always stains with chromatin dyes. Later its contour becomes perfectly regular and it enlarges. Still later two nucleoli are usually found, though as many as six may occur. In the ova of medium size (.272 mm.) the nucleoli are conspicuous bodies and may measure 10 μ in diameter. A body similar to the nucleolus in stain and size was noticed in the cytoplasm. At first it was taken for a centrosome, but as it showed no structure whatever the first hypothesis had to be abandoned. Subsequently it was noticed that one of the two nucleoli moves toward the nuclear membrane and passes through it (Fig. 13). When it reaches the cytoplasm it does not stay there, but moves toward the follicular wall (Fig. 16). It is probable that the migrating nucleolus passes out of the follicle, as it can be seen in all parts of the cytoplasm. The actual process of passing through the follicular wall has not been noticed, but bodies which answer to the description of the nucleolus are occasionally encountered between the follicles. Such migrating

nucleoli are by no means uncommon, as many as six have been noticed in one young ovary. The direction of migration is not predetermined. In retrogressive ova its shape is greatly affected. It may be elongated, crescent-shaped, irregular, or hollow.

2. *Retrogression in Class 1.*

The size limits of the fish vary from 16.5 to 29.5 mm. in total length. The form index averages 4.62 and the fin ratio is 1.16. The germ cells all derived from primordial ova in this stage are anywhere in size between primordial germ cells and medium-sized ova. However, the larger-sized ova are affected first. The first perceivable sign of retrogression appears to be a darker staining zone in the cytoplasm surrounding the nucleus. Later this zone gradually disappears, but at the same time the cytoplasm loses its affinity for stain and appears bleached. The follicular wall, which is one layer of cells thick in the normal ova, now becomes disorganized and it may form two or more layers of cells which may form strands and migrate into the yolk (Fig. 15). The nucleus appears to be affected last. It shrinks, becomes irregular, and disintegrates together with the yolk mass. After the ovum has been entirely absorbed remnants of the follicle remaining for some time witness to the destruction. Some such atrophied follicles may contain several isolated cells which no doubt are the remains of the ingrowing follicular strands. Sooner or later these remnants are also resorbed (Fig. 25).

The retrogression is further characterized by the fact that the epithelium of the ovarian cavity shows no signs of being unfavorably affected. On the contrary it appears very active. It has been noticed, particularly when the ova are greatly reduced by disintegration, that new primordial germ cells originate in the proximity of the epithelium of the ovarian cavity. These could not be traced to cell division, nor could they be traced to preexisting smaller primordial germ cells; the source of these cells is the epithelium itself.

It appears probable that any cell in the epithelium is capable of transforming into a germ cell. All stages of such transformation are encountered in the epithelium as will become apparent from Figs. 17–24. The epithelial cells are small with relatively little

cytoplasm; the nucleus is elongated and may be more or less irregular in contour. The chromatin is granulated and an ill-defined nucleolus can occasionally be noticed. The nuclei take a dark stain and contrast strikingly with the germ cell nuclei. The first sign of such metamorphosis appears to be the activity of the chromatin in which the granules become coarser, forming more or less into lumps. This phenomenon is closely followed by the growth of nucleus and cytoplasm. The elongated and irregular contour becomes regular and the stain loses its density. The final product of such transformed epithelial cells can not in any way be distinguished from primordial germ cells (Figs. 1–2). As these cells increase in size they passively move from the epithelium into the cortex of the ovary to develop into follicles. Fully formed germ cells do occur in the epithelium, which will be discussed later. The transformation of epithelial cells into germ cells is by no means a rarity. It is a common occurrence in all phases of ovarian activity of the females belonging to class 1. Occasionally a place in the epithelium may be found where all stages of such transformation occur in the same field of the microscope. Such a condition is found in Fig. 12. At least six definite stages (*a–f*) are shown in the figure which range from epithelial to completely formed germ cells.

It will be remembered that the epithelium of the ovarian cavity originates from the peritoneal lining of the fusing gonads, and that no germ cells whatever enter into its composition at any time.

The formation of germ cells from epithelium of the ovarian cavity is by no means confined to *Xiphophorus helleri* among the teleosts. Similar observations have been made by Hoffmann (1886) and Böhi (1904) on salmon, Wallace (1904) on *Zoarces viviporus*, Philippi (1908) on *Glandichthys januarius, et al.*

After the transformation process of the epithelium cells into germ cells was discovered, it became apparent that such transformation may occur at any place in the ovary directly from the free cells of peritoneal origin (Fig. 15, *tpc*). The process is the same in principle as described above.

3. *Retrogression in Class 2.*

The general appearance of females of this class is not materially different from class 1. The total length varies between 14.2 and 29.6 mm. The form index averages 4.53 and the fin ratio is 1.17. There are generally fewer primordial ova in the ovary than in the previous class and hardly any one of them appear normal. The most important characteristic of this class lies in the fact that the epithelium of the ovarian cavity is relatively inactive.

The epithelium of the ovarian cavity is no longer surrounded by germ cells, nor are there many at any other place. Even the stroma is very sparingly represented. The ovary looks empty. The outer ovarian epithelium, although intact, shows similar signs of inactivity. Blood vessels are the most prominent structures in the ovary in this class of disintegration (Fig. 26).

4. *Retrogression in Class 3.*

The size limits in this class vary from 18.4–65.0 mm. in total length. The average form index is 4.43 and the fin ratio is 1.84. From Table I. it will be seen that the form index of this class is much closer to that of the male than to that of the female, and that the fin ratio is decidedly that of a young male. In fact, in some of the specimens in this class the anal fin has advanced considerably in the process of transformation. These facts are evidently significant and they will be referred to in connection with the differentiation of the male sex.

Conditions are not less significant as regards the ovary. The outer epithelium of the ovary has been either completely resorbed or else is in advanced stages of resorption. Ova of all stages have left nothing but inconspicuous traces of former presence. Nothing but the epithelium of the ovarian cavity and the oviduct has withstood the destructive process (Fig. 28). The epithelium has shrunk considerably and is by no means of the same appearance in all cases. It looks inactive in some and active in others, although proliferation of germ cells is not apparent. Judging from the appearance of the ovarian remains, it can be said that the renewed activity of the epithelium in later stages of retrogression is not simultaneous with degeneration, but begins afterwards. Remains

of degenerating follicles may be found in the body cavity. Occasionally such remains may assume activity and form cords of various sizes, which may persist for a considerable time.

The fatty tissue which commonly surrounds the gonads in fishes increases immensely during the retrogression. In fact, in the later phases of disintegration of the ovary special care must be taken to discover the remains of the ovary within the fatty mass. The fact that it contains an abundance of large blood vessels leads one to suspect that it might play some rôle in the disintegration process. Although stroma cells have been noticed among the fatty cells, at no time do the latter engulf, surround, or even come in contact with the disintegrating ova. Whatever physiological rôle they play as regards the fate of the germ cells can not be discovered by observation. Experimentally it has been noticed that the fatty tissue increases immensely within two days after injury to the reproductive system.

TABLE I.

Stages in Development.	Averaged Data.		Limits.
	Form Index.	Fin Ratio.	Total Length of Fish in Mm.
Indifferent	6.26	1.0	0 – 9.3
Normal Immature Females	5.02	1.13	9.3 – 16.9
Retrogression in Class 1	4.62	1.16	16.9 – 29.5
Retrogression in Class 2	4.53	1.17	14.2 – 29.6
Retrogression in Class 3	4.43	1.84	18.4 – 65.0
Normal Mature Females	5.34	1.28	26.7 – 80.0
Early Tubule Formation	5.17	1.16	9.3 – 13.5
Middle " "	4.84	1.27	12.5 – 18.6
Late " "	4.64	1.74	15.7 – 51.5
Mature Males	4.20	4.25	31.6 – 84.4

V. EARLY SEX-DIFFERENTIATION OF THE MALE.

The testis of *Xiphophorus helleri* belongs to the acinus type in which seminiferous tubule formation is greatly modified. Sex-cords and tubules are formed from peritoneal cells exclusively. Such tubules give rise to the acini by transformation of epithelial cells into germ cells. The early sex-differentiation of the male is principally a process of tubule formation, and thus it may be divided into early, middle, and late stages of tubule formation.

1. *Early Stage of Tubule Formation.*

The size limits of the young male vary from 9.3 to 13.5 mm. in total length. The average form index is 5.17 and the fin ratio is 1.16, which differs very little, if any, from that of the female in similar stage of development.

The gonads, like those of the female, are far apart at the beginning of sex-differentiation. Gradual approach follows until they meet medially below the air bladder to be separated only by the mesentery. Unlike the ovary, the testes remain permanently paired. The mesentery is well supplied with blood vessels, which at this stage supply the gonads also. There is no difficulty whatever in distinguishing the testis from the indifferent gonad even in early phases of sex-differentiation. It will be remembered that such difficulty does exist in the case of the female. A comparison of Figs. 1 and 2 will make this clear. Even in much earlier stages (Fig. 3) this similarity exists. It may be that the indifferent stage is an early female stage.

Although I have not counted the germ cells in various stages of development, it is nevertheless apparent that the number of the primordial germ cells in the young testis is appreciably less than in the young ovary. Germ cell division was not seen and the cells usually remain isolated. The important rôle in the differentiation of the male is not played by the primordial germ cells, but by the peritoneal cells. The latter proliferate abundantly, and instead of being evenly mingled with the primordial germ cells, cause a segregation of the latter at the periphery of the testis, while the peritoneal cells occupy the center and inner margin (Fig. 30). This aggregation may be known as the sex-cord and, as will be seen in the next stage, is a preparation for tubule formation. In this stage also some of the peritoneal cells are investing the primordial germ cells. Such activity would indicate follicle formation, which is not an adult male structure.

2. *Middle Stage of Tubule Formation.*

The size limits of animals in this stage vary from 12.5 to 18.6 mm. in total length. The form index is 4.84 and the fin ratio is 1.27.

The primordial germ cells play no perceptible rôle in this stage.

They remain on the outer periphery of the gonads, apparently inactive. All activity is vested in the cells derived from the peritoneum. These cells form at first a solid cord, the sex-cord, in the central part of each gonad, parallel with the longer axis of the animal. The cord formation in the young testis is antero-posterior, but as the elongation of the gonad is mostly anteriorly, the cord formation later follows the elongation of the testis. The cord resembles very much a tubular gland except that there is no lumen at first. The peritoneal cells place themselves side by side with their apices pointing centrally in the sex-cord, which in a transverse section appears rosette-like (Fig. 31). These cells increase considerably in size and number, which causes the increase in diameter of the cord and the origin of a central lumen; or, in other words, transforms the cord into a tube which is the testicular cavity or sperm duct. It must be pointed out that there is no indication whatever that any of the germ cells participate in the formation of the sperm duct.

3. *Late Stage of Tubule Formation.*

The size limits of animals in this stage vary from 15.7 to 51.5 mm. in total length. The wide range is significant in that it shows great variation in the time of sex-differentiation in the male. The average form index of this period is 4.66 and the fin ratio is 1.74, which is decidedly male. There is a slight increase in diameter in the third ray in the preceding stage, but, strictly speaking, transformation of the anal fin into an intromittent organ or gonopod does not begin before the present stage is reached. The gonad is decidedly differentiated as testis before the anal fin is affected. The first indication of the transformation of the anal fin into a gonopod is the thickening of the third ray. This is shortly followed by the elongation of the third, fourth, and fifth rays until approximately twice the length of the original fin is reached. The rest of the rays—*i.e.*, the first, second, and seventh to eleventh—are not subject to any particular change.

The gonad has increased appreciably in size owing primarily to the proliferation of the peritoneal derivatives and only secondarily to increase of the primordial germ cells. Most commonly the testis is butterfly-shaped in the transverse section, but may be very

irregular, depending upon its previous history. The primordial germ cells are almost invariably in nests, which would indicate that cell division has taken place; mitotic figures, however, have not been seen. These nests of germ cells always occupy the periphery of the testis and have no connection with the tubules.

The sperm duct or the main tube of the testis of the previous stage is now branched and as development proceeds the branches become subdivided (Figs. 32 and 38–39). Each testis may be roughly compared to a bunch of grapes with the sperm duct as the main stem. The tubules thus produced are radially arranged with their apices toward the periphery of the gonads. This tubule formation is peculiar to *Xiphophorus helleri* and closely related genera and, as far as the writer knows, has not been described before.

If one of these radial tubules is examined, it will be found that it consists of an inner epithelial layer and an outer homogeneous membrana propria (Fig. 33). This membrane is very thin and contains a very few small nuclei. The epithelial cells are cuboidal to columnar in shape, according to stage of development. In size and staining reaction the cells are intermediate between peritoneal and primordial germ cells. In more advanced stages of development the tubules show various stages of germ cell metamorphosis (Figs. 33–36). The apex may become separated from the rest of the tubule by a constriction and assume a spherical form; the tubular lumen has become obliterated by the growth of cells which are now spherical in shape and differ from the primordial germ cells at the periphery of the testis only in position. The next portion of the tubule shows cells in a less advanced stage; the lumen may still be present, the cells and particularly the nuclei are smaller in size and less regular in shape; the cells take a darker stain than the apical cells and there is no delimiting membrane from the more basal cells. The proximal portion of the tubule may differ in no essentials from the young tubule except that the cells show an increase in size. Later on the acini separate completely from the tubule, but for a long time the radial arrangement is maintained (Figs. 37–38).

It will be remembered that in the ovary definitive germ cells are formed from two different sources:

1. The epithelium of the ovarian cavity.

2. Free cells in the ovarian cortex of peritoneal origin. Similar conditions are found in the testis. The first and by far the most common source is the epithelium of the tubules described above. The second source, precisely as in the female, is the free peritoneal cells of the testicle. The method of transformation differs in no essentials from that found in the ovary. Such germ cells move toward the periphery of the gonad, multiply, and eventually become spermatocysts.

The variability in the development of the testis in this stage can not be overlooked. They occur too often and too regularly to be classified as haphazard abnormalities. The testis of the young fish from 15–20 mm. in total length is a very definite structure. Its definite shape, its regular contour, and its distinct bilaterality are characteristic (Fig. 38). In cases where transformation occurs, however, the gonad varies in shape, is irregular in contour, and, what is more striking, is only partially bilateral. Generally the posterior part of the testis shows more or less complete bilaterality, while the anterior part is in a fused state, thus producing a bifurcated testis (Figs. 40 and 41). There is no indication whatever that the typical shape of the testis occurring in those specimens that transform early becomes irregular and the bilateral testis becomes fused as development advances. On the contrary, the typical testis maintains its definite shape during the life of the animal, while the irregularly shaped testis gradually becomes regular as development proceeds, and at the time of spermatogenesis the shape of the testis is more or less definite in all. All of the above facts lead to the inference that the bifurcated testis has resulted from the epithelium of the ovarian cavity after complete disintegration of the ovules. In fact, all stages can be found between the epithelium of a degenerated ovary and a testis.

The formation of the extra-testicular sperm duct belongs to this stage, although some traces of it are already found in early and middle stages of tubule formation. Generally speaking, the male and female ducts are homologous. Both begin from an anterior and a posterior primordium. In both the posterior primordium is more advanced in origin and extent of formation than the anterior primordium. In both the anterior portion of the duct is of peritoneal origin, and this is in all probability true of the posterior por-

tion as well. The formation of the lumen of the posterior part of the duct is the same in both sexes. The anterior portion, however, presents some slight differences. In the male the lumen is formed by the extension of the intra-testicular sperm duct into the posterior primordium. The ends of the two sperm ducts meet slightly back of the testis and fuse to form a single duct. Not only is the origin and method of formation of the male and female ducts similar, but the young sperm duct and the young oviduct are identical in structure. Specialization takes place only later in development.

VI. Late Sex-differentiation of the Female.

The smallest normal mature female found in my material is 26.7 mm. in total length. The largest is 80.0 mm. long, which can be considered a full-grown specimen. The average form index is 5.34 and the fin ratio is 1.28.

Externally the sexually mature female can be recognized by the appearance of a dark spot on each side in the region of the pelvic fins. This is not due to pigmentation of the dermis, but of the peritoneal lining of the body cavity above and posterior to the ovary. In males and young females the peritoneal lining is silvery white or slightly pigmented. As the female approaches sexual maturity the pigment increases immensely and can be seen through the translucent body wall as a black spot. The name "Trächtigkeitsfleck" has been applied to denote this characteristic, but since it is maturity instead of "Trachtigkeit" that causes its appearance, a more appropriate name would be puberty spot. The size of the spot varies directly with the size of the ovary and the period of gestation. It is very conspicuous at the time of the birth of the young and this probably explains the origin of the German name.

A female with a puberty spot always contains mature ova. There may be from one to nearly one hundred in number, depending upon the age of the fish. They are of a brilliant amber color, measuring on the average 1.6 mm. in diameter. The ova lie in the cortex of the ovary and often occupy the entire space between ovarian lumen and ovarian wall (Fig. 27). Each is contained in a follicle consisting of a single layer of cuboidal cells. The follicles are surrounded by a very slightly developed theca folliculi which emerges indistinctly into the surrounding stroma. Imma-

ture ova of all sizes can be found all over the ovary, but most commonly in the region of the epithelium of the ovarian cavity. Yolk is deposited at the time the ova have reached medium size. At this time the young egg has a beautiful alveolar structure in section due to the presence of oil globules which have been dissolved by reagents.

The epithelium of the ovarian cavity of the adult *Xiphophorus helleri* differs in no essentials from that of *Glaridichthys* described by Philippi (1908). Owing to the encroachment of the adult ova it has been thrown into numerous folds which occasionally are not unlike the villi of the small intestine. The characteristic depressions in the epithelium of the ovarian cavity called "Delle" by Stuhlmann (1887) are very prominent in *Xiphophorus helleri*. Each depression is an invagination of the epithelium of the ovarian cavity into the ovarian substance directed toward an egg. It serves two purposes: admission of the sperm into the ovum and creation of a place of rupture for the escaping young. Its formation begins with rather young ova, slightly below medium size, but is not completed until the time of fertilization.

The oviduct of the adult female is very short. It consists of three layers: an outer muscular, a middle connective tissue, and an inner epithelial. The latter is thrown into folds which may either project into the lumen like villi or overlap and form pockets. According to Philippi (1908) these pockets serve as hiding places for spermatozoa in which they may remain and maintain vitality for over 160 days. The oviduct enters the uro-genital sinus at its anterior border, projects backward to open immediately in front of the aperture which is located slightly back of the anus.

VII. Late Sex-differentiation of the Male.

Here are comprised the stages in which spermatogenesis takes place. The size limits vary from 30 to 84.4 mm. in total length. The form index of the sexually mature male averages about 4.2, while the fin ratio is 4.25.

The various stages of spermatogenesis are rather difficult to describe owing to the fact that cell division has thus far evaded the writer's observation. Of course, the difficulty lies mostly in the proper application of terminology and not so much in the changes that take place.

As has been pointed out, the acinus or spermatocyst is the end product of the tubule formation and the beginning of spermatogenesis. The definitive germ cells in the acini approach the size and appearance of the primordial germ cells before spermatogenesis begins. At this period the nuclei of these cells measure from 7.2 to 9 μ in diameter. This is the largest size they ever reach. The cell dimensions are hard to measure because the entire acinus presents the aspect of a syncytium (Fig. 36). On account of the size and what is to follow, it would seem proper to term these cells primary spermatocytes. The next thing that happens to the acini is the doubling of their volume and the number of their cells. Evidently cell division has taken place. The picture has changed radically (Fig. 37, *ssc*). The nuclei no longer show a clear aspect with more or less thread-like chromatin; the stain is darker, the chromatin is broken up into a great many rod-like structures; the nuclear membrane is absent and the cytoplasm is greatly diminished. The nuclei now measure on the average 5.96 μ in diameter. This might be considered a secondary spermatocyte stage.

Apparently another division takes place as the number of nuclei doubles. This time there is no appreciable increase in size of the acini. The size of the nucleus, however, has diminished to 2.5 μ, which is practically one half of the previous stage. The color has not changed, but the cytoplasm is reduced to a minimum; in fact, it can hardly be demonstrated with general methods of staining (Fig. 37, *sds*). It is probable that we are dealing with the spermatid stage.

In the next stage the metamorphosis of the spermatids into spermatozoa is quite evident. For the first time the acinus becomes luminated as the developing spermatozoa move toward the very thin, homogeneous wall of the acinus, where they form a complete layer of one layer of cells thick. The tail differentiates and the spermatozoa are all oriented so that the tail is free in the lumen. The head, or rather the nucleus, gradually decreases in size as it elongates. The final dimensions are 1.35 x 2.55. There is also a decrease of size in the entire acinus or spermatophore, which measures about 50 μ in diameter, or half that of the secondary spermatocyte stage. The heads of the spermatozoa are so closely pressed together that they appear like a single layer of epithelial cells (Fig.

37, *sph*). They stain very dark and with iron-hemotoxylin the cross-section of a spermatophore presents a solid dark ring. The tails are fairly long and are twisted altogether in a sort of spiral. Occasionally a belated spermatozoan has failed to get "in line" and remains in the lumen among the tails. The ripe spermatophores occupy the central portion of the testis and are found in every part of the sperm duct. In this condition they are discharged and reach the female genital tract, where by the action of the ovarian secretion the outer membrane is dissolved and the spermatozoa swim freely in the oviduct or ovarian cavity.

The question naturally arises as to the meaning of the primordial germ cells at the periphery of the testis (Figs. 37–38, *ppgc*). In comparison with the rest of the germ cells, they appear inactive. It can not be said with certainty that their number decreases with age, nor is there any reliable sign that they take part in spermatogenesis. In a few diseased males which were affected by a tumor growth in the tail region the testis was affected to a marked degree. All the spermatophores, mature and immature, were broken up with the spermatozoa free in the lumen. Formation of new acini was going on in all parts of the testis, but the peripheral primordial germ cells showed no more activity than in the normal. This seems to lend evidence that the primordial germ cells do not furnish a source of definitive germ cells.

It seems to be necessary to emphasize at this place that no retrogressive development of any sort is encountered in the normal process of sex-differentiation in the male of *Xiphophorus helleri*.

The sperm duct is slightly longer than the oviduct because the testis is more anterior in position. It consists of three layers: an outer muscular, a middle connective tissue, and an inner epithelial. The cells of the epithelium are flagellated. The contour of the epithelium is very regular. Just as in the case of the female, the sperm duct enters into the uro-genital sinus to open just in front of the aperture. In both male and female the urethra opens into the anterior part of the uro-genital sinus. The uro-genital aperture lies at the base of the gonopod and does not enter into it.

It will be remembered that the transformation of the anal fin into an intromittent organ or gonopod begins during the late stage of tubule formation. The first noticeable thing in such meta-

morphosis is the thickening of the third ray; in fact, it should be said rays, as all of them are paired and lie side by side (Figs. 44 and 45). As the thickening of this ray continues, the third, fourth, and fifth rays elongate until approximately twice the length of the original fin is reached (Figs. 48 and 49). Next the tips of the last-named rays form knob-like projections which are to be transformed into hooks in later development (Fig. 47). As a general rule, spermatogenesis begins at this stage of the transformation of the anal fin, although variations are not uncommon. The first, second, and sixth to tenth rays are subject to no special changes and remain rudimentary. The third ray, which has increased immensely in thickness medially, forms an S-like structure apically and the distal arm projects out on the ventral margin of the fin to form a copulatory hook. This hook is reinforced by the tip of one of the members of the fourth rays, which has turned ventrally, and we shall call it the ventral part of the fourth ray. The same kind of a hook is formed on the opposite margin of the fin by the union of the two members of the fifth ray (Fig. 46). Furthermore, the third and the dorsal part of the fourth ray form two rows of symmetrically placed "teeth" which project backwards and outwards, forming a hollow external groove in the former which leads toward the tip of the gonopod. There are approximately 8 "teeth" on each side of the third ray and 9 in the fourth. The two members of the fifth ray in the secondary growth regions, anterior to the hook, have fused and broadened latero-dorsally to form a concave groove on the dorsal margin of the gonopod. All the apical modifications of the rays are supposed to be for copulation and transmission of the spermatophores from the male to the female. At the time the anal fin is metamorphosed into a gonopod the spermatophores are formed and ready for discharge.

Besides the gonopod the beautiful sword of the *helleri* male and the pelvic fins are secondary sex characters and must be considered here. Both of them, however, start their transformation somewhat later than the anal fins.

The sword shows the first signs of development shortly before spermatogenesis begins. It is formed from the ventral lobe of the tail fin of the male fish. The ten ventral rays of the caudal fin are involved; most of the elongation falls upon the sixth to

tenth and the utmost length is reached by the eighth ray. In the adult male the length of the sword approximates the total length of the fish. The pigmentation of the sword is very striking. The middle rays are from greenish to orange in color; this is bounded on both sides by deep black. The dorsal portion of the caudal fin has a yellowish hue.

The pelvic fins of the male differ from those of the female in the relative length of the rays. The length of the first or anterior ray of the pelvic fin of the male is to the second ray as 1 : 2, while the reverse is true of the female—*i.e.*, as 2 : 1. These differences appear late in sex-differentiation, approximately at the beginning of spermatogenesis.

VIII. Sex-ratios.

The sex-ratio in the immature fishes between the sizes of 10–26 mm. in total length is given in Table II. These limits were chosen because sex is unmistakably established at 10 mm. and normal adult females appear at about 27 mm. in total length. The specimens in this table were taken at random and thus they present the actual conditions in the population.

Table II.

Normal Females.		Retrogressive Females.		Males.		Total.		Percentage.	
No.	Size.	No.	Size.	No.	Size.	Males.	Females.	Males.	Females.
21	10 – 16.7	58	12.5 – 26	44	10 – 26	44	79	36%	74%

It is very clear from the above table that there is a great preponderance of females in *Xiphophorus helleri* in the immature condition—*i.e.*, in the developmental stages falling between 10 and 25 mm. in total length. For all practical purposes the ratio may be reduced to two females to one male.

For sex-ratios in mature fishes the writer is indebted to Dr. A. W. Bellamy. Table III. is compiled from his data. Each record gives the history of an entire brood reared from birth to sexual maturity.

TABLE III.

Record.	No. Born.	Females.	Males.	Unaccounted.	Percent. of Males.
106 *E*	40	1	35	4	94
114 *A*	13	2	11	0	85
106 *D*	81	0	60	21	100
112 *DEF*	106	26	33	47	56
112 *G*	47	27	6	14	18
34 *AB*	12	1	9	2	90
113 *ABC*	17	2	10	5	83
Total	316	59	164	93	75%

From the above tables it is evident that the sex-ratios have suffered reversal from immature to mature conditions; also that the change is very marked. One can perceive that such change could be brought about in two ways: differential viability and sex inversion.

Oxygen consumption experiments have shown (Bellamy, 1922) that the males consume approximately twice as much oxygen as the females. The males are also by far the more active. From these data one might expect to find greater mortality among the males than among the females. This is borne out experimentally. If both sexes of equal chronological age are subject to unfavorable conditions such as weak solutions of potassium cyanide, alcohol, or excesses of temperature, the male invariably succumbs first. With some of these conditions, such as cyanide or alcohol, the females live twice as long on the average as the males and sometimes even longer. That the reversal of the sex-ratio is not due to mortality of the females can also be ascertained by keeping strict records of broods of fishes from birth to sexual maturity. The mortality of *Xiphophorus helleri* under proper conditions is slight, indeed, and whenever death occurs the sex can be established cytologically. The results of such observations are decidedly in consonance with the experimental results and it is perfectly safe to conclude that the female is at least as sturdy as the male.

If the reversal of sex-ratios from immature to mature condition is not due to differential viability, there is only one other possibility, and that is sex inversion. This occurs most commonly in fishes from 16–27 mm. in total length, but may occur at any size from 16 mm. upward.

IX. Inversion of Females.

It will be seen in Table II. that all females between 16.7 and 26 mm. are retrogressive. Normal females do not occur before they reach approximately 27 mm. in total length. The phenomenon of retrogression in *Xiphophorus helleri* is not a seasonal fluctuation, as this species produces young practically every month. Nor is it temporary and reversible, once the ova are affected they can not be rejuvenated. Lane (1909) has described retrogressive ovules in *Lucifuga* and *Stygicola*. In these teleosts the larger and more favorably situated ova cause the disintegration of smaller ovules which are absorbed as food. It is very clear that this is not the case in *Xiphophorus*, for it is the larger ova that are affected first. These may be in a hopeless state of degeneration before the smaller ones show signs of disintegration. My material strongly favors the conclusion that all of the linear descendants of the primordial germ cells disintegrate.

If the retrogressive females transform into males, it is apparent from the above tables on sex-ratios that approximately half of the females become males. It is doubtful whether any of the females of class 1 transform, because the ovarian epithelium is actively proliferating definitive germ cells. The activity of the epithelium is relatively slight in females of class 2 and stops entirely in females of class 3, and therefore one would expect that the prospective males are recruited from the two latter classes. If so, the sum of the females of classes 2 and 3 must approximately equal the sum of females of class 1. This is actually the case, as is evidenced by the following table, in which all of the retrogressive females studied are tabulated.

Table IV.

Class 1.	Class 2.	Class 3.
40	25	10

The question may be raised whether the retrogressive females result in sexual forms. In the first place, no sexless form has been encountered in the 300 fishes used for cytological work unless the females of class 3 be so considered. Careful study shows conclusively that they are not stationary but transitional forms, and

that they are in stages between a completely disintegrated ovary and the origin of the male gonad—*i.e.*, the late stage tubule formation. In the second place, breeding experiments have shown that there are no sterile individuals unless diseased, which, as I have pointed out, are not very common. There are several cases where a fish has been selected as a normal female and placed in an aquarium with a male for breeding purposes and after several months two well-formed males have been found in the aquarium.

Finally the evidence that such retrogressive females may develop into males may be summarized as follows:

1. The sex-ratios described above, in which the ratio is reversed at maturity from what it was in immature stages, favors very strongly the idea of sex-inversion.

2. The occurrence of a bifurcated testis in certain males (see p. 60) which is to be explained as a connecting link between female and male.

3. The irregular contour of the testis and the large and irregular tubules of the presumed arrhenoids (Figs. 40–41) contrast conspicuously with the normal testis (Fig. 38).

4. The condition of the anal fin is further supporting evidence. Making ample allowance for all errors, the average fin ratio of the retrogressive female is far into the domain of the male (Table I.). In fact, the general appearance of such an anal fin suggests the male condition in the stage of late tubule formation, whereas there is nothing more than the remains of the epithelium of the ovarian cavity.

5. The form index and the advanced age of the arrhenoid fish as compared with normal sex-differentiation of the male are additional arguments in favor of sex-inversion in *Xiphophorus helleri*.

The opinion of breeders and fish fanciers has been for a number of years in favor of sex-inversion in *Xiphophorus helleri* and related forms. Their observations have been confined naturally to external features; mainly to form index and anal fin. I have had the opportunity to meet the members of the Chicago Aquarium Club and hear reports on first-hand observations dealing with the problem. Several members of this club have favored me with material of various kinds for which I wish to express my sincere appreciation.

Several reports dealing with sex-reversal in *Xiphophorus helleri* have appeared in *Wochenschrift für Aquarien und Terrarien Kunde*. The following may be cited from No. 17, August, 1920, p. 273: "Eine längere Abhandlung bringt Herr A. Poser über die Umbildung von Weibchen in Männchen bei Helleri. Herr P. welcher ganz der Absicht des Herr Dr. Mertens ist ("BI." 20, No. 13) wird von anwesenden Mitglieder der Erweis erbracht, dass auch bei uns diese Umbilding bei roten H. beobachted wurde. Eine Verwechlung mit anderen Jungtieren anderer Weibchen ist ausgeschlossen, da nur ein Exemplar vorhanden war. Die Umbildung vollzog sich sehr langsam."

X. Comparative.

It may be of interest to submit a brief comparative summary of some of the publications dealing with the instability of sex in animals. This will be done under three headings: sex-inversion in teleosts, sex-inversion in other vertebrates, and the origin of definitive germ cells.

1. *Sex-inversion in Teleosts.*

As far as the writer has been able to ascertain the first report on arrhenoid fishes was furnished by Herzenstein (1891). The report concerned *Gymnocypris potanini* and *Schizopygopsis Güntheri*, both cyprinidont fishes. The observation was based on secondary sex characters—*i.e.*, on the assumption by the female of the secondary sex characters of the male.

Philippi (1904) reported "Ein neuer Fall von Arrhenoidie" in the viviporous teleost *Glaridichthys caudimaculatus*, which he describes as follows: "Ich isolirte anfangs October zwei anscheinend trächtige Weibchen von G. caudimaculatus zwecks besser Beobachtung. Während das eine an 17 October Junge warf, zeigte das andere drei oder vier Tage vor diesen Datum eine Veränderung an der Analflosse, die aber so schwach war, dass ich über ihr Wesen nicht ins Klare kommen konte. Am 17 October war diese Veränderung so weit vorgeschritten, dass sie als schwache, aber deutliche Verlängerung der vorderen Strahlen erkennbar war. Am 7 November war die Analflosse bereits bis auf etwas das Dappelte des Normalen Ausgezogen, so dass die der eines halberwachsenen Männchen in der Form glich."

In 1908 the same author reported three more cases of arrhenoidy, all of which were *Glarydichthys januarius*. In all three the form index was strictly female, while the gonopod was well advanced. After one of them died it was examined microscopically and showed total absence of a gonad, while the duct was typically oviduct. It is clear that this particular fish was in a late retrogressive stage, when it is very difficult to isolate the epithelium of the ovarian cavity. In one of the remaining two Philippi found the following peculiarities: "Makroskopisch liess dieses 3, ausserlich in bezug and die grösse ganz als Weibschen erscheinende Tier 2 nicht miteinander verschmolzene milchweisse Hoden erkennen, in deren einem 2, dottergelbe grosse Eier sich befanden und die beide einem typischem Oviduct Aufsassen."

Newman (1908) reported on a case in *Fundulus majalis*, which he at the time called "A significant case of hermaphroditism in fish." As the specimen had advanced toward maleness in morphology and behavior during the period of observation, and as *F. majalis* is decidedly bi-sexual, the case is apparently one of sex-inversion.

2. *Sex-inversion in Other Vertebrates.*

The work by Brandt (1889) on birds is of considerable interest. The name "arrhenoidie" was coined to replace "Hahnenfedrigkeit." It deals mostly with the domestic fowl, but observations on game birds, etc., are not uncommon. Birds that have laid eggs and have otherwise appeared and acted like females have been observed to assume the appearance and behavior of the male sex. Such changes occur most commonly with senility, but this is not necessary, for birds of one year of age are known to transform. It has been noted that some abnormalities, such as a solid or blind duct, occur, but there are many with no such apparent causes. Cytologically various stages of disintegration of ovarian structures and new formation of apparently testicular tissues go hand in hand. The differentiation of seminiferous tubules first begins as solid cords which luminate and may develop from one to several layers of epithelium. Such tubules show signs of spermatogenesis; no spermatozoa, however, have been encountered.

Work of somewhat similar character has been described by Pearl

and Curtis (1909), Boring and Pearl (1918) on domestic fowl, and Pearl and Surface (1915) on the cow.

An interesting case of complete sex-inversion in Tritons was reported by Champy (1921). Here the inversion was from male to female, which is generally considered uncommon. The Triton in question was used for breeding purposes as a male. The transformed Triton had a typical oviduct and juvenile ovary containing numerous oöcytes which were in the process of yolk formation. Champy's conclusion is as follows: "Ensomme nous avons chez un animal adulte l'etat ovarien d'une femalle jeune. Etant Dannee l'histoire anterieure de l'animal, il n'est pas doutex que nous avons un cas d'interversion sexuelle totale."

Frogs and toads have long been known to show peculiarities as to sex conditions. Many of such abnormalities or "hermaphrodites" have been described by various observers. Lately Crew (1921) has shown that all of the "abnormalities which have been recorded can be so tabulated that the first case most nearly approximates to the normal female and the last the typical male, with respect to the nature of both primary and secondary sex characters. Thus arranged it is seen that the cases furnish an almost complete series of gradations which range from an individual almost completely female to one almost completely male, and that the conditions found readily appear to be merely graded stages of a single process."

Witschi ('21), after a thorough investigation of the sex conditions in frogs, concludes as follows: "Die Froshzwitter sind stets genetische Übergangsformen zwischen den reinen Geschlechtern (Übergangshermaphroditen), und zwar geht die Entwicklung von weiblichen zum männlichen Geschlecht."

3. *Origin of Definitive Germ Cells.*

It was pointed out in the foregoing pages that in the teleost a number of investigators have observed the origin of the definitive germ cells from peritoneal derivatives. Such conditions have been reported by Hoffmann (1886), Böhi (1904), Wallace (1904), and Philippi (1908).

Swingle (1921) reported that the lineal descendants of the primordial germ cells in *Rana catesbeiana* degenerate. As to the

origin of the definitive germ cells, he says: "In the interval between the first and second larval sexual cycle following the degeneration of large numbers of maturation cells the gonad becomes filled with small cells which, because of their size, nuclear structure, and staining capacity, appear as transition stages between mesothelial cells (germinal epithelium and sex-cord elements) and true germ cells. The later history shows them to be germ cells, but their origin is open to two interpretations and is not as clear as could be desired. The writer considers these cells as small germ cells descendants of the primordial sexual elements, and not as transformed germinal epithelium elements, but admits that the evidence from his material is equally strong for the germinal epithelium viewpoint."

Clearer cut results have been obtained by Firket (1920) in the albino rat. The primordial germ cells disintegrate and have disappeared entirely in the testis of the albino rat from the tenth to the fifteenth day after birth. The origin of the definitive germ cells in the albino rats, according to Firket, are as follows: "At the time the first spermatogonia appear they are easily recognizable by the texture of their nuclei and are very numerous. This has been shown very distinctly in the same species by Hoven. Let us insist that those spermatogonia can only be derived from the small epithelial cells, as they are at this stage the only type of cells present in sufficient numbers in the sex-cord. The spermatogonia must be called 'secondary germ cells.'"

It was stated previously (p. 47) that the data presented in this paper comprise only a part of the study of the sex problem of *Xiphophorus helleri*. Particular interest is attached to the experimental work in progress at the present time. Closer cytological study of the material is imperative to elucidate difficulties encountered in cell division, chromosome composition, etc. A discussion of the bearing of the present data on the theory of sex will be postponed until the completion of the entire problem.

XI. Summary and Conclusions.

1. At birth the young fish measure on the average 8 mm. in total length and are on the verge of sex-differentiation.

2. The gonads of the indifferent stage are paired and widely

apart, suspended in a peritoneal sac immediately below the air bladder.

3. The indifferent gonad consists of two kinds of cells: primordial germ cells and cells of peritoneal origin. Both are evenly distributed in the gonad.

4. At 10 mm. in length sexes are distinct. In females the indifferent gonad develops into an ovary without any marked morphological changes of the gonad except that the primordial germ cells gradually enlarge and become oöcytes.

5. In males distinct changes take place in which the germ cells and the peritoneal cells are segregated, the former on the periphery of the gonad, the latter occupy the median and inner portions of the gonad.

6. The paired gonad in the female fuses to form a single ovary. The median surface of the fusing gonads become the ovarian cavity.

7. In the male the gonads remain paired permanently.

8. Both the oviduct and the sperm duct are formed from two sources: posteriorly from the peritoneal lining of the body cavity and anteriorly from the gonad.

9. All females from about 12.5–26 mm. in total length are subject to retrogression. To all appearances all primordial germ cells disintegrate. Definitive germ cells come from peritoneal cells.

10. Completely disintegrated ovaries have been found with epithelium of the ovarian cavity appearing from very inactive to very active. The anal fin in such cases is in early stages of transformation into a gonopod.

11. The fate of the primordial germ cells in the male is uncertain. Definitive germ cells originate from peritoneal cells.

12. Bifurcated testes occur in a large number of cases, which are supposed to originate from a completely disintegrated ovary.

13. Sex-ratios are reversed from immature to mature condition.

14. Differential viability is in favor of the female—*i.e.*, the female is at least as sturdy as the male.

15. The material decidedly favors sex-inversion in *Xiphophorus helleri*. This takes place most commonly in immature fishes, but may occur in adult animals.

16. The transformation of the anal fin into a gonopod takes

place after differentiation of the testis in normal males, but before differentiation in transformed males.

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EXPLANATION OF PLATES.

All the drawings have been made with the aid of an Abbe Camera. The lenses used were of Zeiss make and the magnifications follows the description of each figure.

PLATE I.

FIG. 1. Transverse section of indifferent gonad of a specimen 9 mm. in total length. × 1000.

FIG. 2. Transverse section of an early normal ovary. × 1000.

FIG. 3. Transverse section of an indifferent gonad of an embryo 3.2 mm. total length. × 1000.

Symbols:

bc Binucleated germ cell.
ee External epithelium of gonad.
ln Labulated nucleus of germ cell.
m Mesentery.
mo Mesovarium.
p Peritoneum.
pc Peritoneal cell.
pgc Primordial germ cell.
rc Red blood cell.
yg Yolk globules.

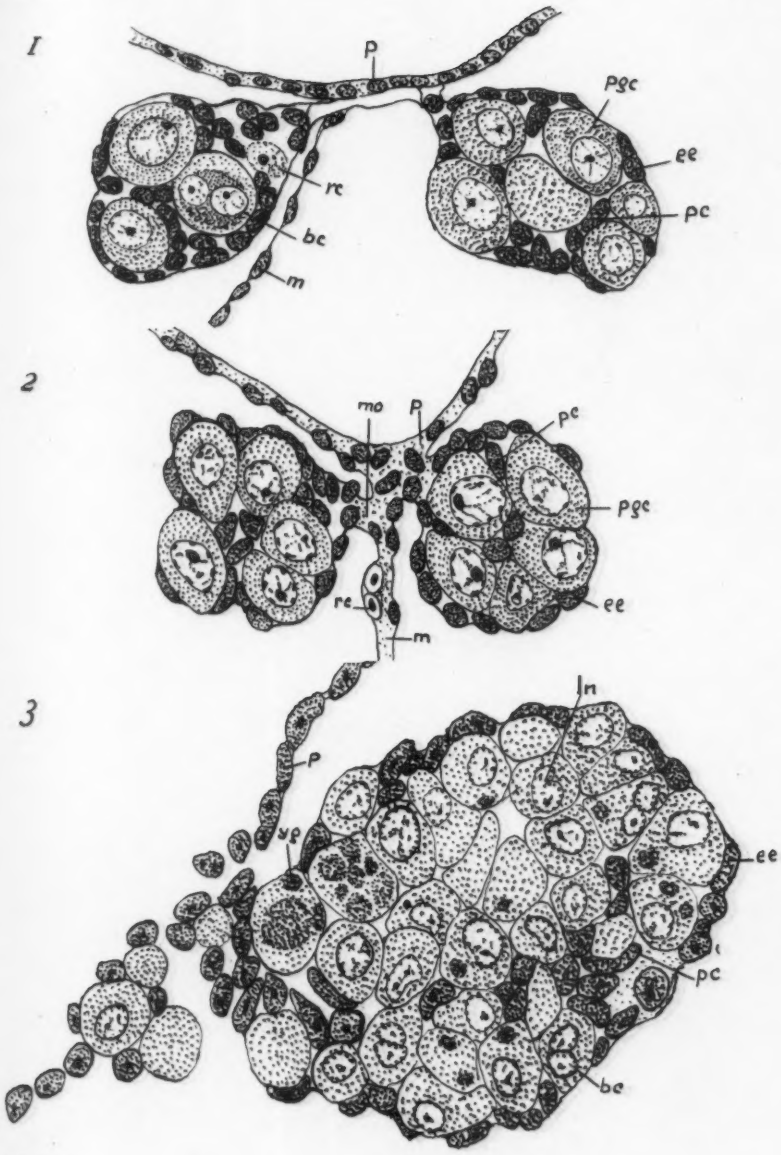

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Plate II.

Note: All drawings of this plate are made from a preparation of a young normal ovary of a single individual.

Figs. 4–5. Transverse section of anterior and posterior parts of ovary. × 115.

Fig. 6. Transverse section of the anterior primordium of oviduct through the anterior region. × 115.

Fig. 7. Transverse section of the anterior primordium of oviduct through middle region. × 115.

Fig. 8. Transverse section of the anterior primordium of oviduct through posterior region. × 115.

Fig. 9. Transverse section of central portion of oviduct showing a solid cord of cells. × 115.

Fig. 10. Transverse section of posterior primordium of the oviduct through the anterior region. × 115.

Fig. 11. Transverse section of posterior primordium of the oviduct through the posterior region. × 115.

Symbols:

ee External epithelium of gonad.
epo Epithelium of ovarian cavity.
ft Fatty tissue.
lo Lumen of oviduct.
m Mesentery.
mo Mesovarium.
nt Nest of primordial germ cells.
o Ovum.
oc Ovarian cavity.
od Oviduct.
p Peritoneum.
pgc Primordial germ cells.
ppo Posteriorly extending portion of ovary.
rt Rectum.
thm Thickened portion of mesovarium.
ub Urinary bladder.
ur Ureter.
urt Urethra.

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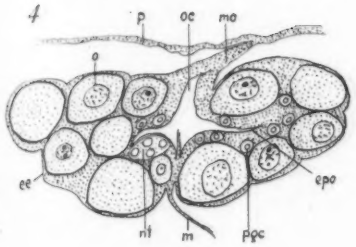

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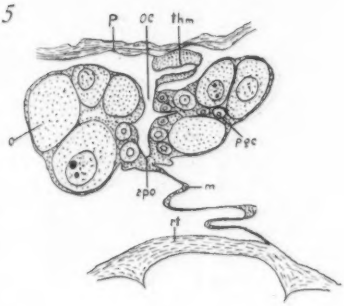

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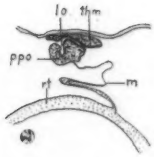

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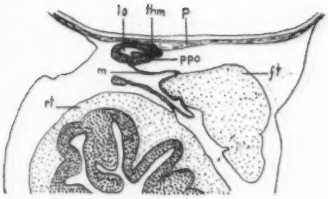

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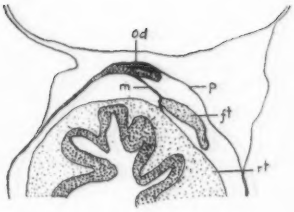

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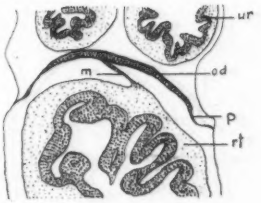

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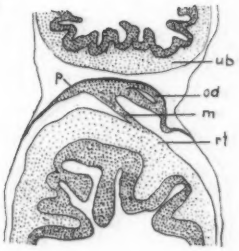

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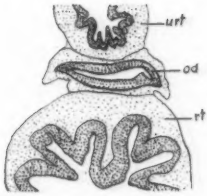

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PLATE III.

FIG. 12. Portion of transverse section of epithelium of the ovarian cavity showing transformation of epithelial cells into definitive germ cells. × 1000.

FIG. 13. Section of medium-sized ovum to show migration of nucleolus from nucleus into cytoplasm. × 410.

FIG. 14. Portion of a transverse section of a young female between anterior and posterior primordia of oviduct showing no central duct formation. × 1000.

FIG. 15. Portion of a section of a disintegrating ovum showing disorganized follicular epithelium. × 1000.

FIG. 16. Section of medium-sized ovum to show migration of nucleolus towards periphery of ovum. × 416.

Symbols:

a–f	Various stages of transformation of epithelial cells into definitive germ cells.
bv	Blood vessel.
ec	Epithelial cell.
fe	Follicular epithelium.
m	Mesentery.
mno	Migrating nucleolus.
no	Nucleolus.
oc	Ovarian cavity.
p	Peritoneum.
rt	Rectum.
thf	Theca folliculi.
ub	Urinary bladder.
y	Yolk.

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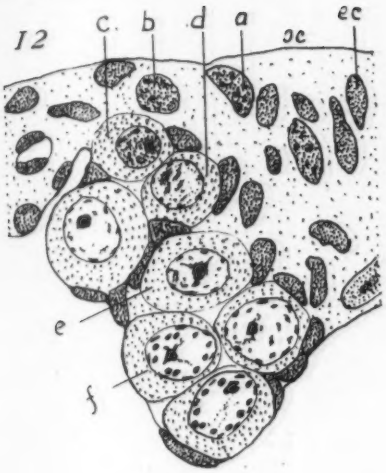

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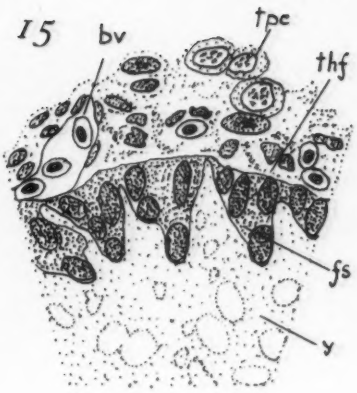

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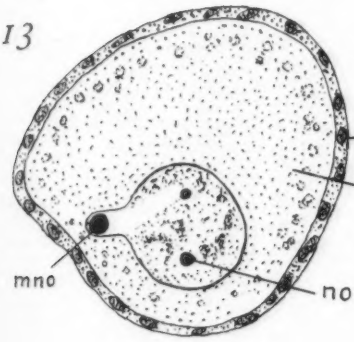

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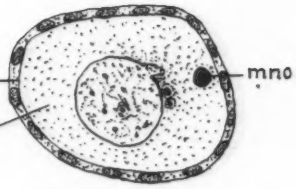

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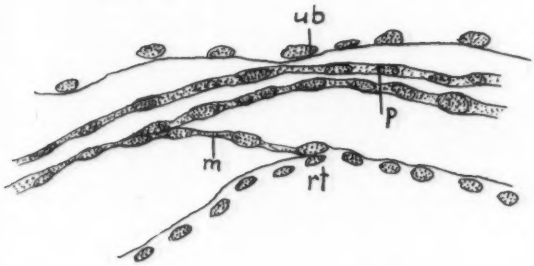

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PLATE IV.

FIGS. 17–24. Portions of transverse section of the epithelium of the ovarian cavity showing transformation of epithelial cells into definitive germ cells. × 1600.

Symbols:

a–f Various stages of transformation of epithelial cells into definitive germ cells.

oc Ovarian cavity.

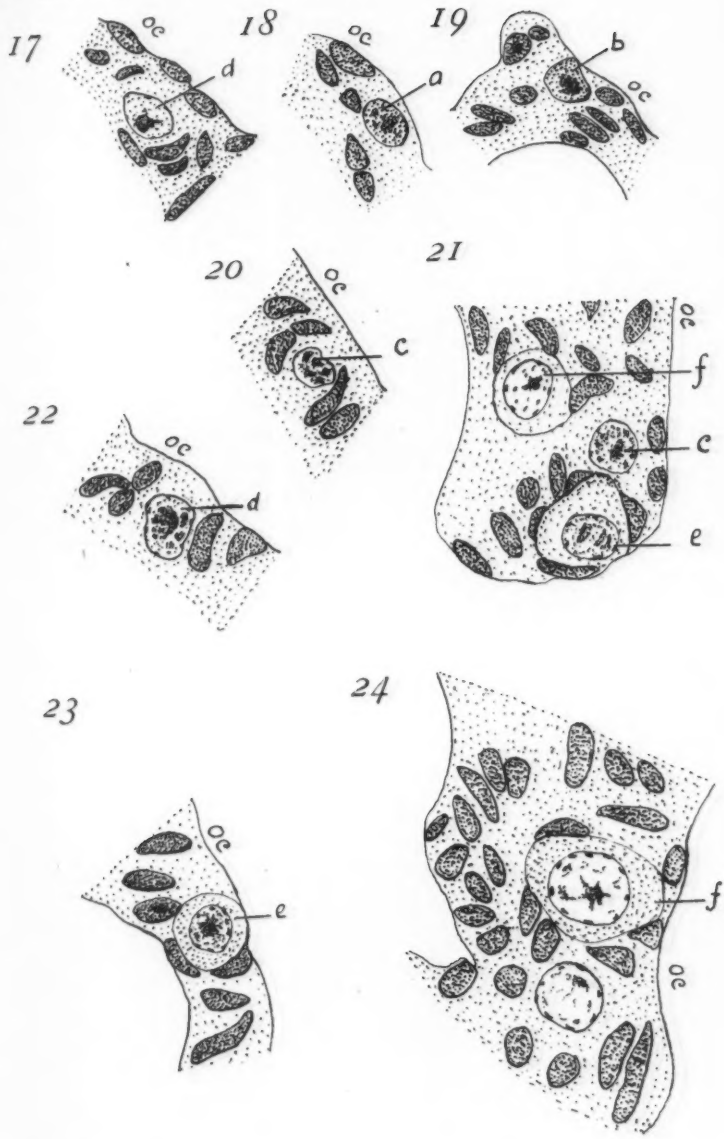

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PLATE V.

FIG. 25. Transverse section of retrogressive ovary in class 1. × 80.
FIG. 26. Transverse section of retrogressive ovary in class 2. × 140.
FIG. 27. Transverse section of normal mature ovary. × 22.5.
FIG. 28. Transverse section of retrogressive ovary in class 3. × 205.
FIG. 29. Transverse section of oviduct of ovary in Fig. 28. × 115.

Symbols:

bv	Blood vessel.
ctc	Connective tissue coat.
dfe	Disorganized follicular epithelium.
dno	Disintegrating ovum.
ee	External epithelium of gonad.
epc	Epithelial coat.
epo	Epithelium of ovarian cavity.
fed	Follicular epithelium of disintegrated ovum.
oc	Ovarian cavity.
mc	Muscular coat.
o	Ovum.
oo	Oöcyte.
ro	Remains of disintegrated ova.

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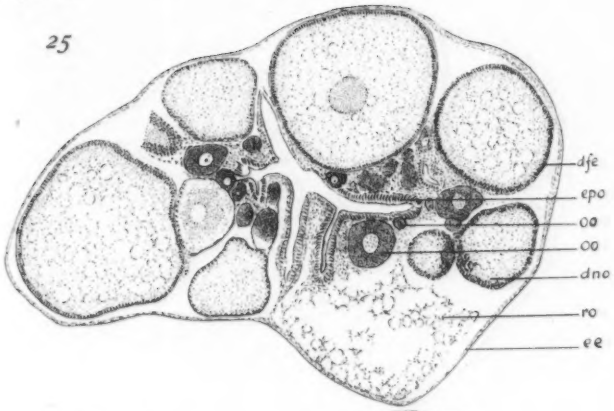

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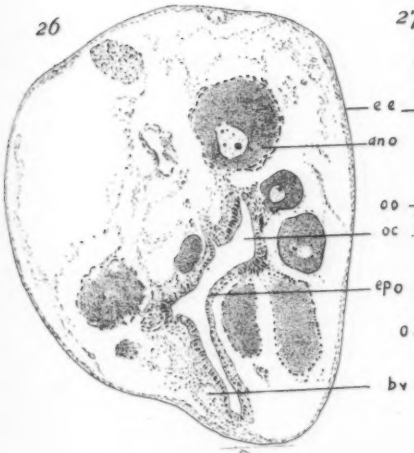

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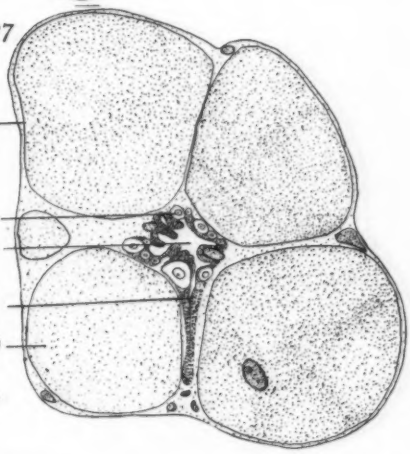

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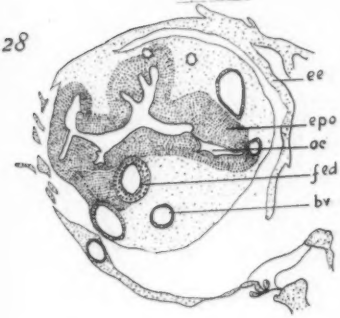

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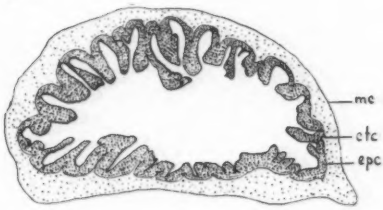

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PLATE VI.

FIG. 30. Transverse section of testis in early stage of tubule formation. × 1000.

FIG. 31. Transverse section of testis in middle stage of tubule formation. × 1000.

FIG. 32. Transverse section of testis in late stage of tubule formation (early phase). × 1000.

Symbols:

ee	External epithelium of gonad.
m	Mesentery.
mch	Mesorchium.
nt	Nest of primordial germ cells.
p	Peritoneum.
pc	Peritoneal cells.
pgc	Primordial germ cells.
rc	Red blood cell.
sc	Sex cords.

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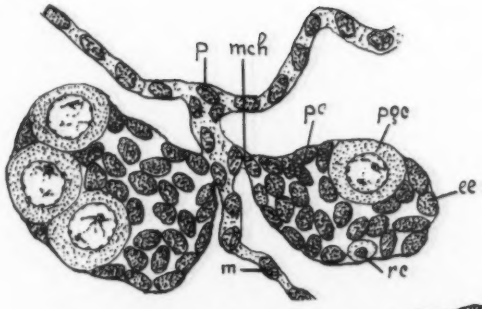

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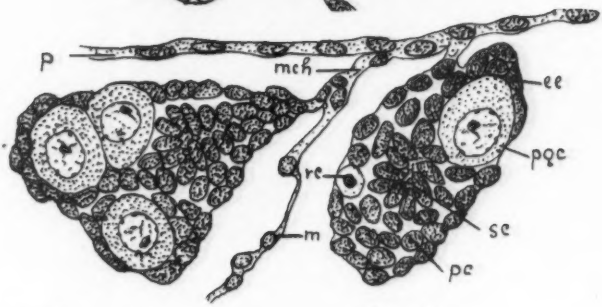

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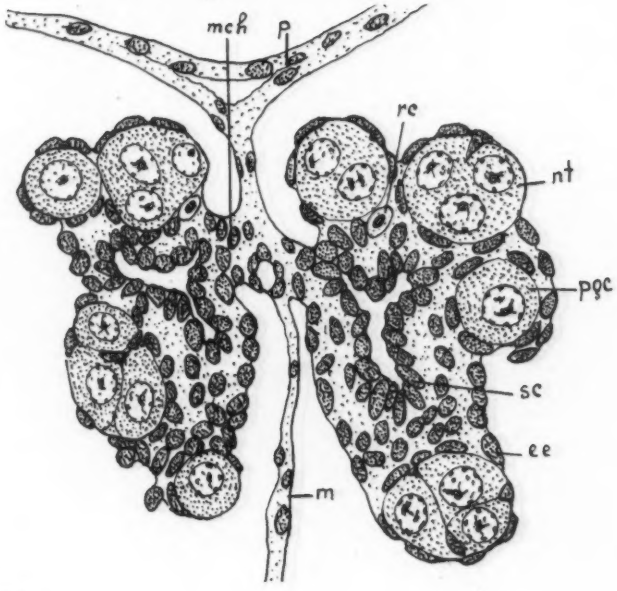

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Plate VII.

Figs. 33–36. Transverse section of radial tubules to show transformation of tubule epithelium into definitive germ cells. × 1600.

Symbols:

et	Epithelium of tubule.
fgc	Fully formed germ cells.
mp	Membrana propria.
lrt	Lumen of radial tubule.

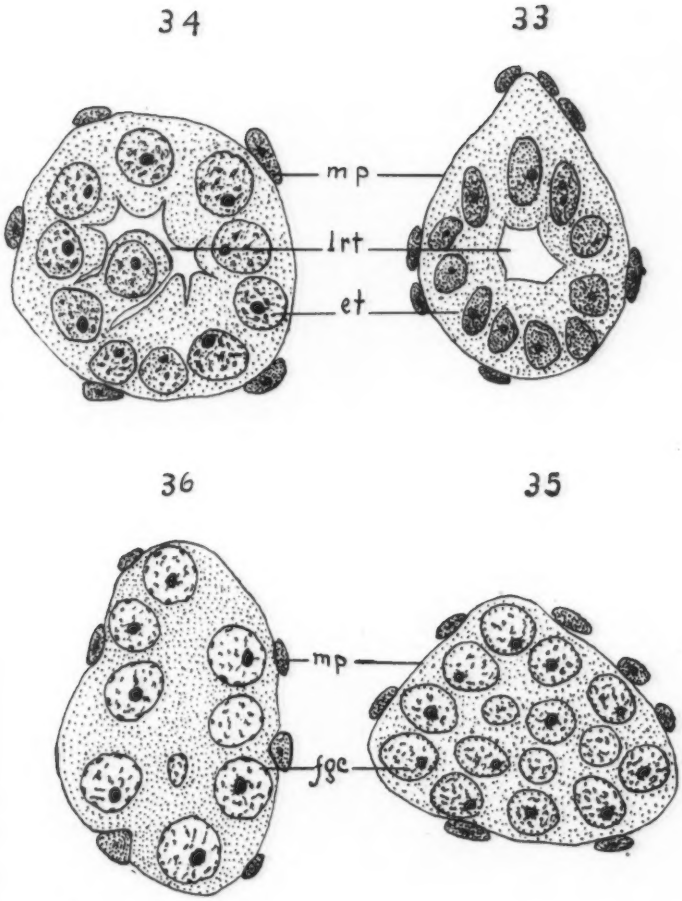

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PLATE VIII.

FIG. 37. Portion of transverse section of adult testis showing spermatogenesis. × 410.

Symbols:

ppgc	Primordial germ cells on periphery of testis.
sds	Spermatids.
sph	Spermatophore.
sps	Spermatozoa.
ssc	Secondary spermatocytes.

37

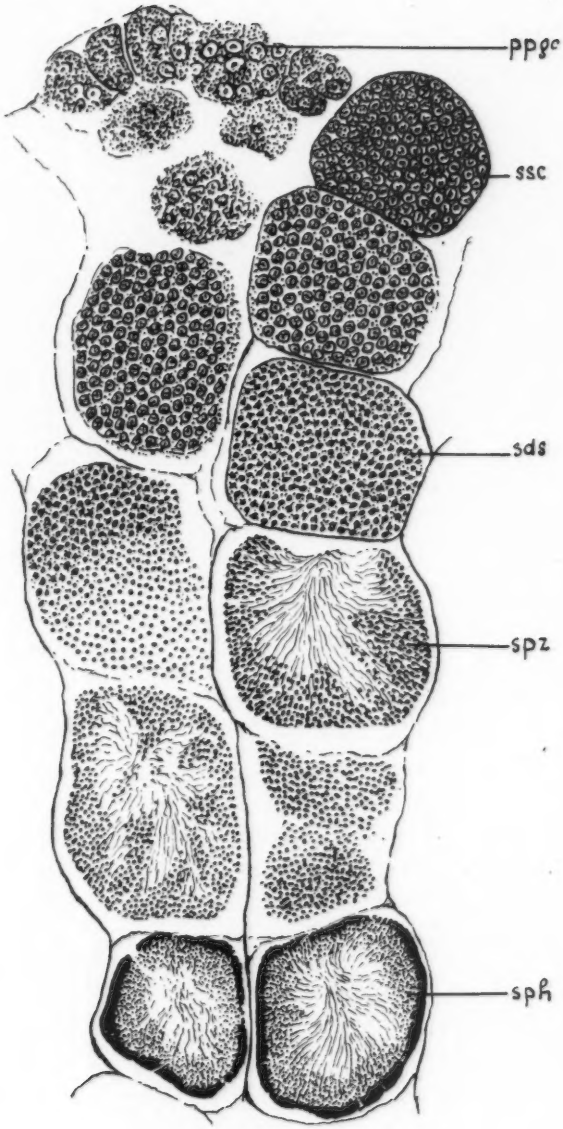

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Plate IX.

Fig. 38. Transverse section of testis in late stage of tubule formation (late phase). × 135.

Fig. 39. Portion of transverse section of testis in late stage of tubule formation to show branching tubules. × 205.

Fig. 40. Transverse section of bifurcated testis, anterior part. × 467.

Fig. 41. Transverse section of bifurcated testis, posterior part. × 467.

Fig. 42. Anal fin of indifferent stage. × 55.

Fig. 43. Anal fin of adult female. × 10.

Fig. 44. Transverse section of adult gonopod from level *A–A*, Fig. 46. × 25.

Fig. 45. Transverse section of adult gonopod from level *B–B*, Fig. 46. 25.

Symbols:

ac	Acinus or spermatocyst.
bsc	Branch of sex cord.
bv	Blood vessel.
d4r	Dorsal branch of fourth ray.
ee	External epithelium of gonad.
fep	Fin epithelium.
m	Mesentery.
mch	Mesorchium.
ntc	Nest of definitive germ cells.
p	Peritoneum.
ppgc	Primordial germ cells in periphery of testis.
sc	Sex cords.
sp	Spoon-like structures.
spd	Sperm duct.
t3r	Teeth of third ray.
t4r	Teeth of fourth ray.
v4r	Ventral branch of fourth ray.
1r–10r	First to tenth rays of anal fin.

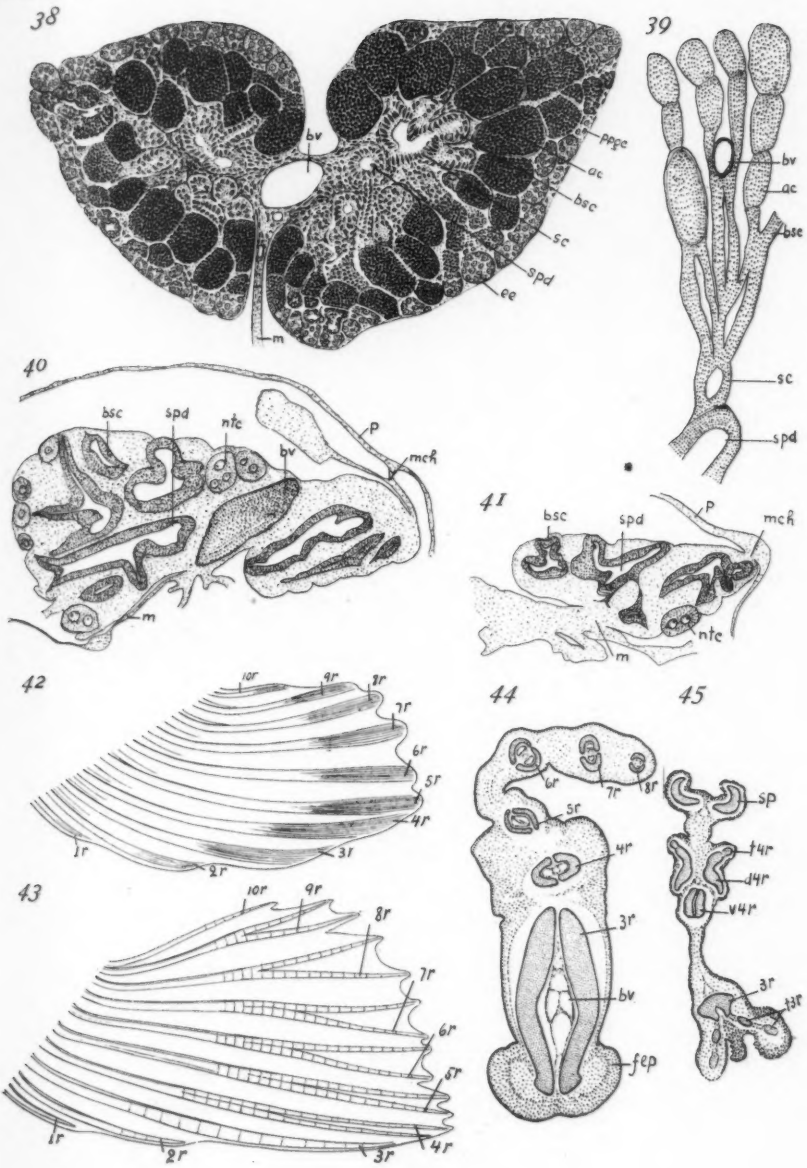

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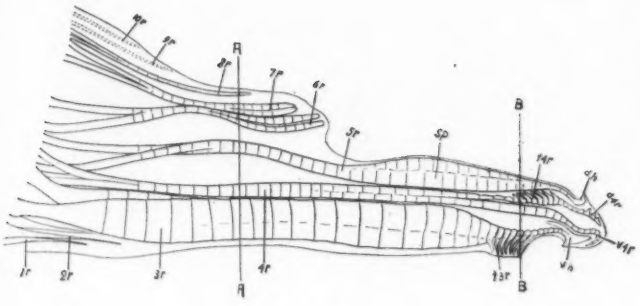
Plate X.

Fig. 46. Adult gonopod. × 17.

Fig. 49–47. Gonopod in various stages of metamorphoses. × 17.

Symbols:

dh	Dorsal copulatory hook.
d4r	Dorsal branch of fourth ray.
kn	Knobs.
sp	Spoon-like structure.
t3r	Teeth of third ray.
t4r	Teeth of fourth ray.
vh	Ventral copulatory hook.
v4r	Ventral branch of fourth ray.
1r–10r	First to tenth rays of anal fin.



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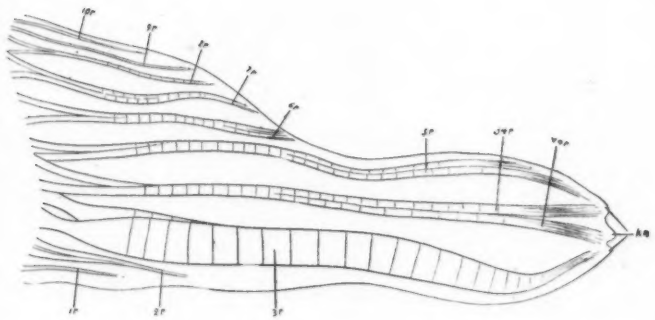

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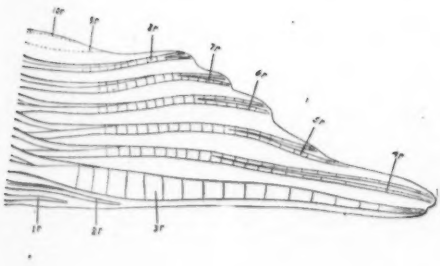

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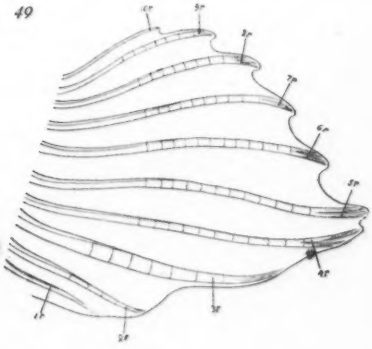

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